MATERNAL IODINE DEFICIENCY AND PRENATAL BRAIN DEVELOPMENT

by

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VOLUME II

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Fig. 1 Josef Warkany and "The Poor in Iodine".

.....That such a complicated disorder as endemic cretinism could be wiped out without explanation of the intricate mechanisms involved in its pathogenesis may be disappointing to some scientific purists but consoling to those who like shortcuts in the prevention of congenital disorders.

Josef Warkany (1971)

Fig. 2 The clinical spectrum of endemic cretinism. Modified from Delange et al. (64).

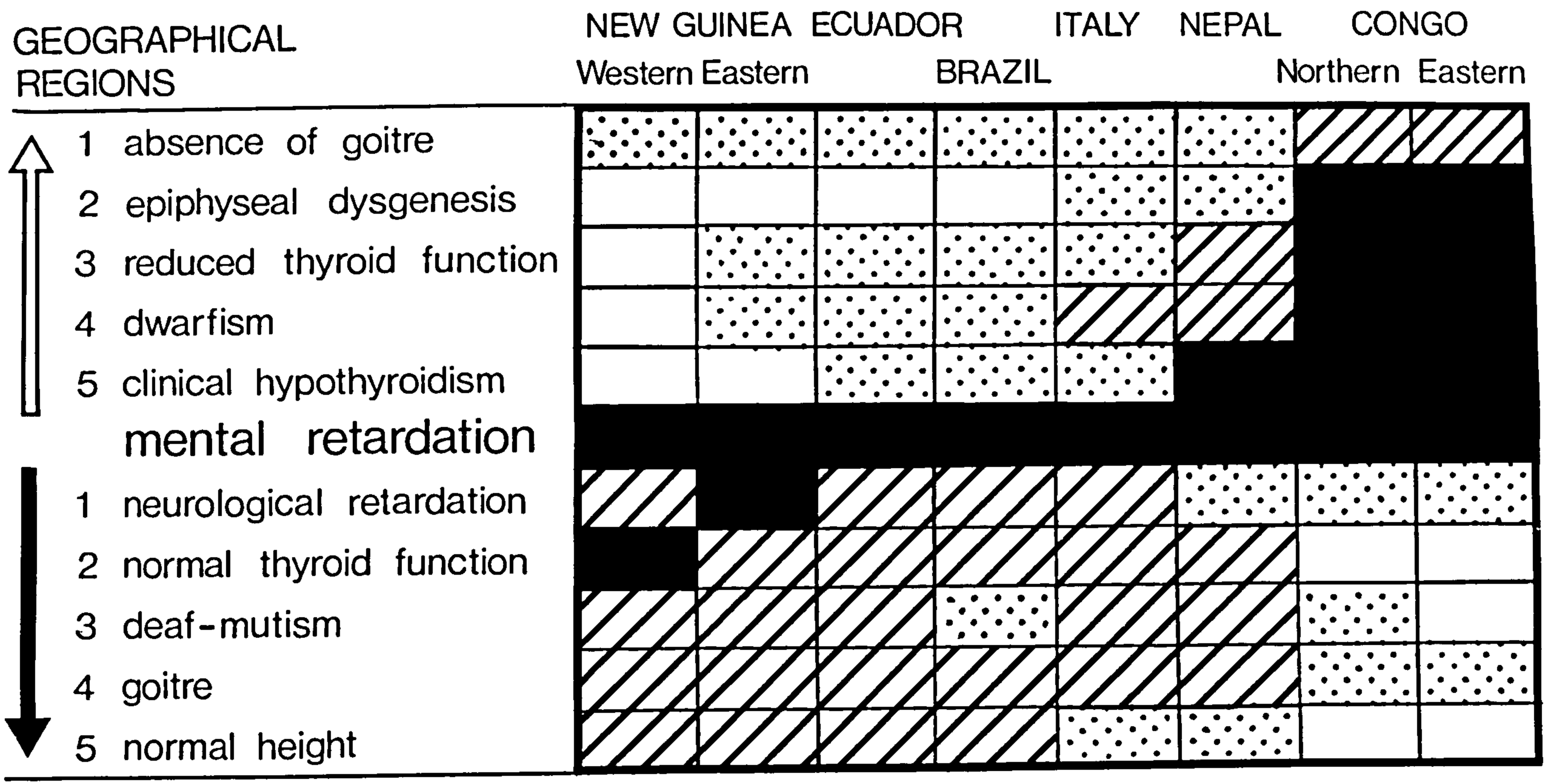

GEOGRAPHICAL REGIONS
NEW GUINEA
Western
Eastern
ECUADOR
BRAZIL
ITALY
NEPAL
CONGO
Northern
Eastern
1 absence of goitre
2 epiphyseal dysgenesis
3 reduced thyroid function
4 dwarfism
5 clinical hypothyroidism
mental retardation
1 neurological retardation
2 normal thyroid function
3 deaf-mutism
4 goitre
5 normal height
△ MYXŒDEMATOUS
▼ NEUROLOGICAL
None
Some
10–50
Most
>50 per cent
All

Fig. 3 Principal features of endemic cretinism.

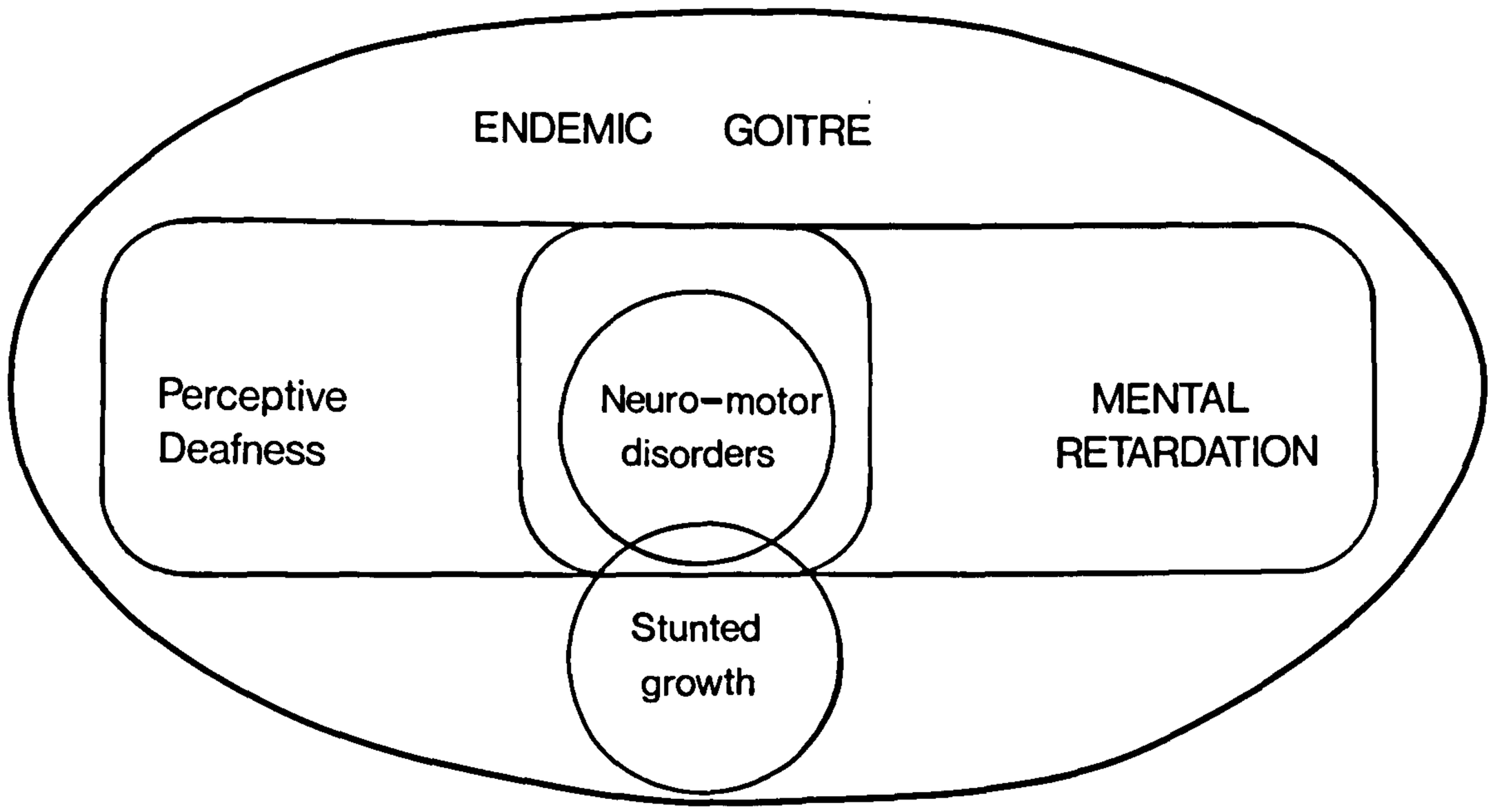

ENDEMIC GOITRE
Perceptive Deafness
Neuro-motor disorders
MENTAL RETARDATION
Stunted growth

Fig. 3

Fig. 4 Iodisation and deaf-mutism in Switzerland.
Drawn from data of Wespi (284).

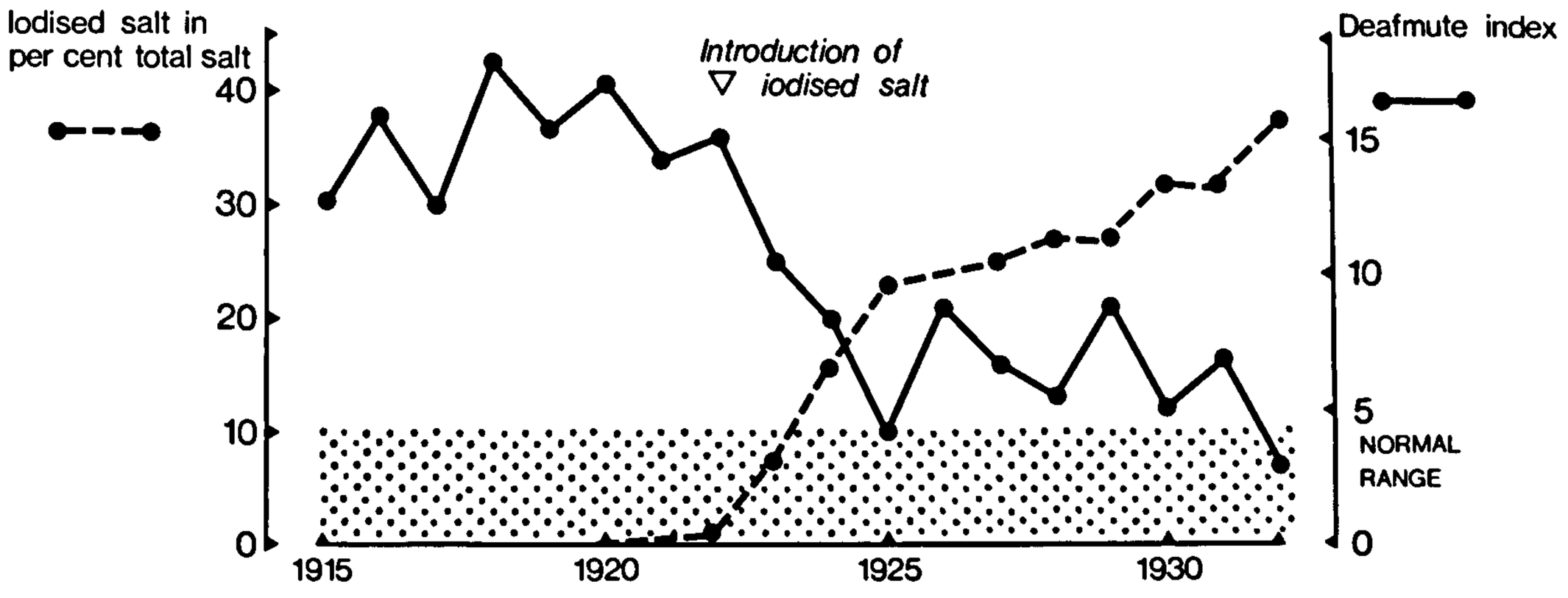

Iodised salt in per cent total salt
40
30
20
10
0
Introduction of iodised salt
Deafmute index
15
10
5
0
NORMAL RANGE
1915
1920
1925
1930

Fig. 4

Fig. 5 Prevention of endemic cretinism in the Jimi Valley.

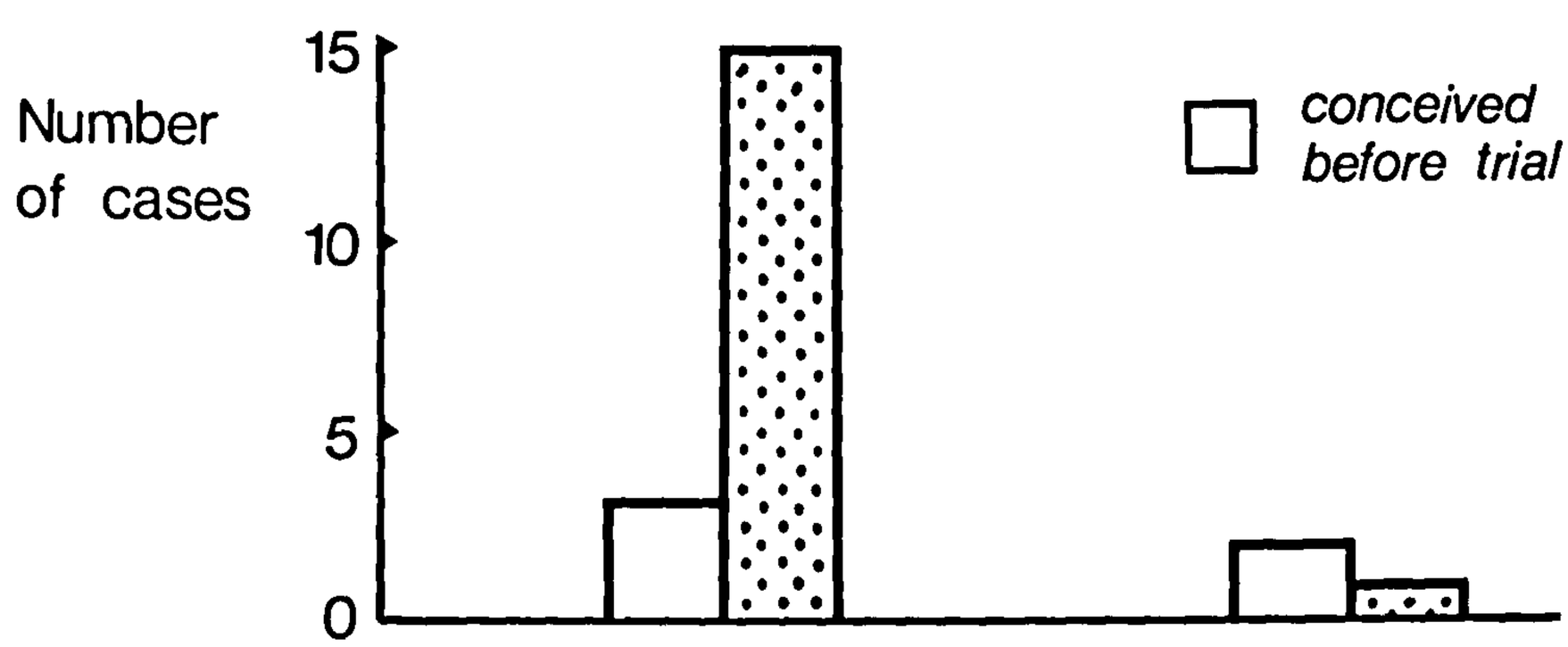

MOTOR RETARDATION, DEAFNESS AND SQUINT.
Number of cases
15
10
5
0
conceived before trial
MOTOR RETARDATION AND DEAFNESS ONLY.
Number of cases
15
10
5
0
conceived after trial
SALINE CONTROLS (688)
IODISED OIL TREATED (687)

Fig. 5

Fig. 6 Relation of serum T_4 to serum TSH in endemic goitre. After Chopra et al. (38).

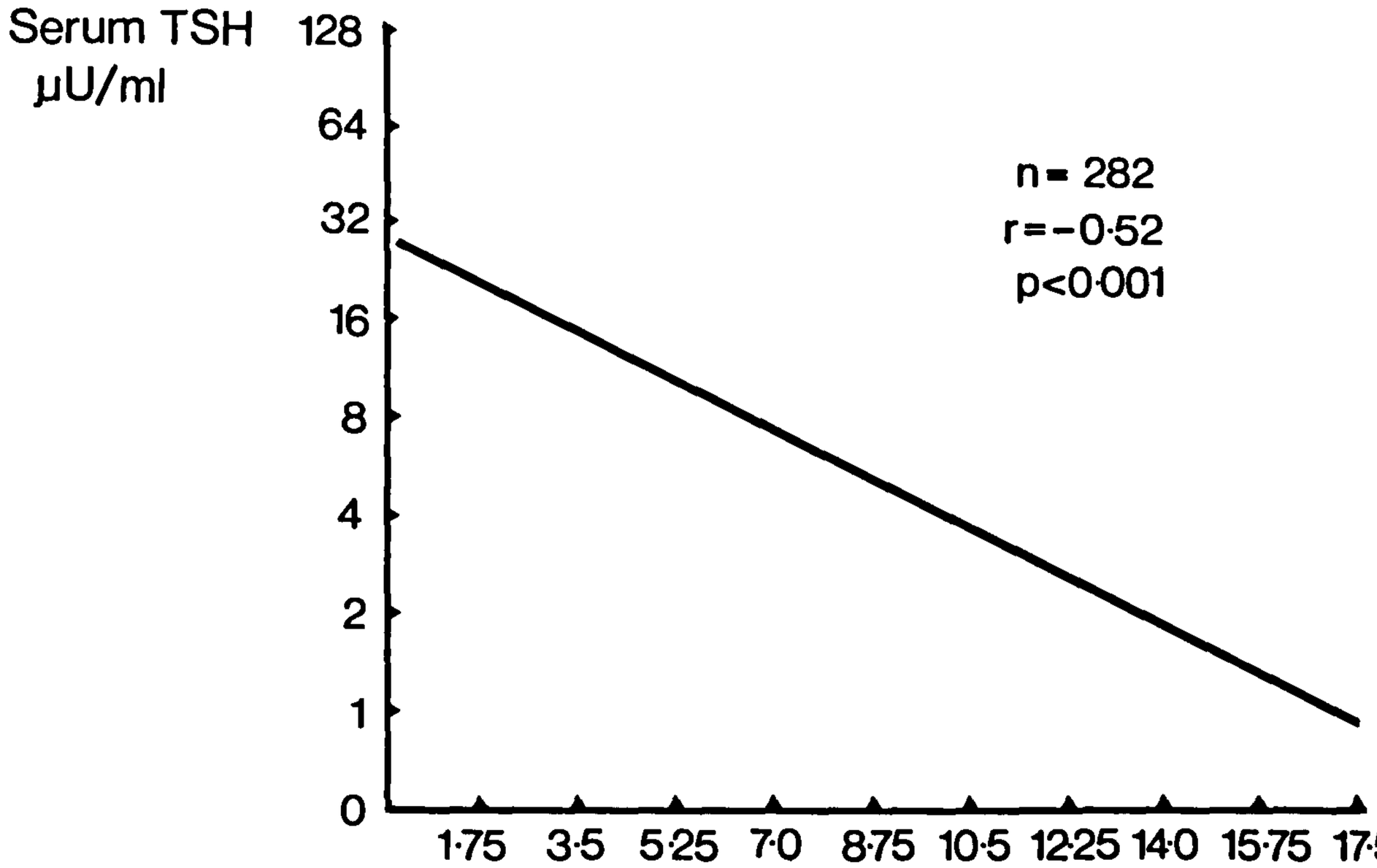

Serum TSH
µU/ml
128
64
32
16
8
4
2
1
0
n = 282
r = −0·52
p<0·001
1·75
3·5
5·25
7·0
8·75
10·5
12·25
14·0
15·75
17·5
Serum T4 – µg/100ml

Fig. 6

Fig. 7 Development of the HPT axis in man. Modified from Fisher et al. (115).

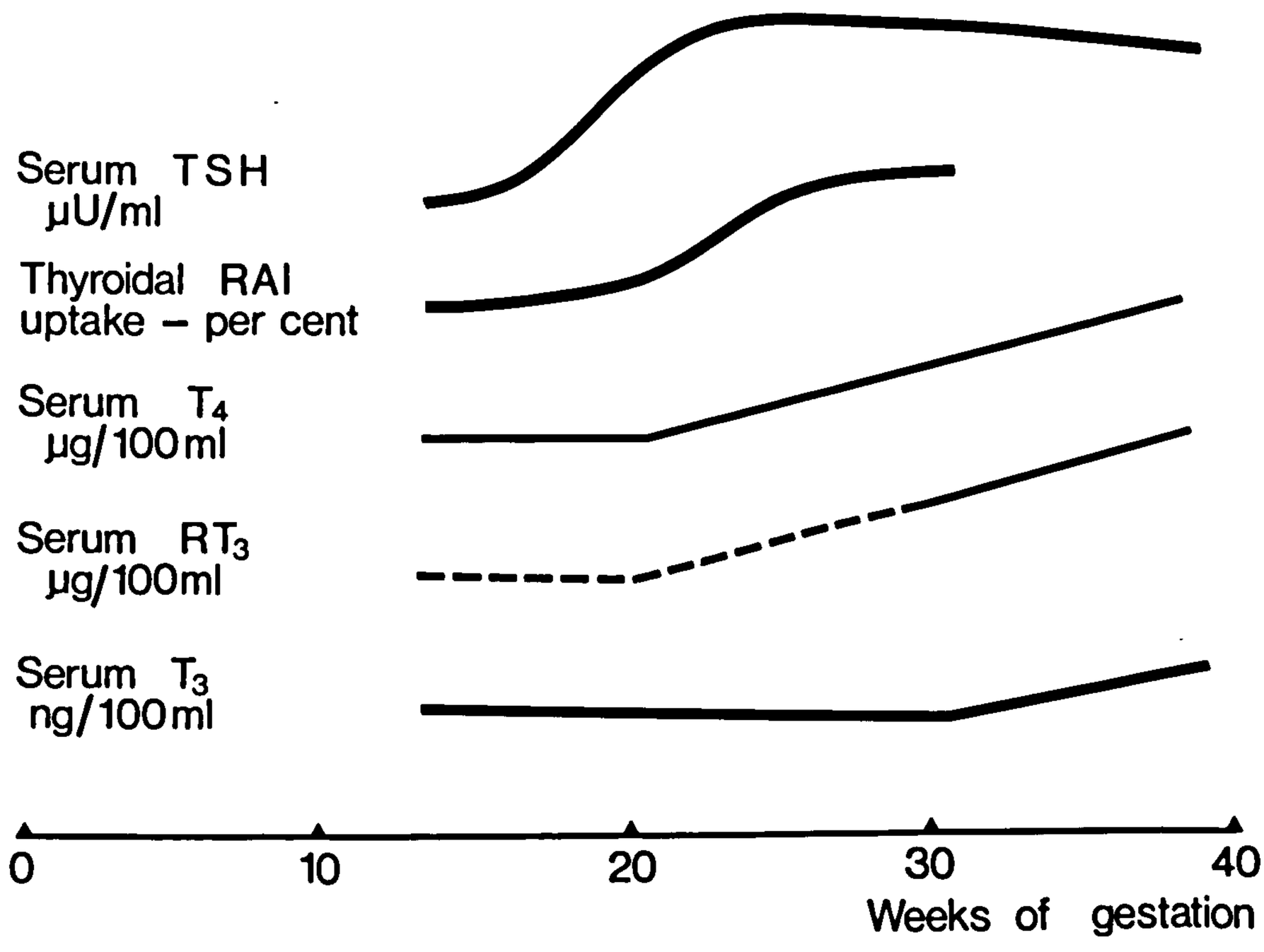

Serum TSH
µU/ml
Thyroidal RAI
uptake – per cent
Serum T_4
µg/100ml
Serum RT_3
µg/100ml
Serum T_3
ng/100ml
0
10
20
30
40
Weeks of gestation

Fig. 7

Fig. 8 Development of the HPT axis in the rat. Modified from Fisher et al. (115).

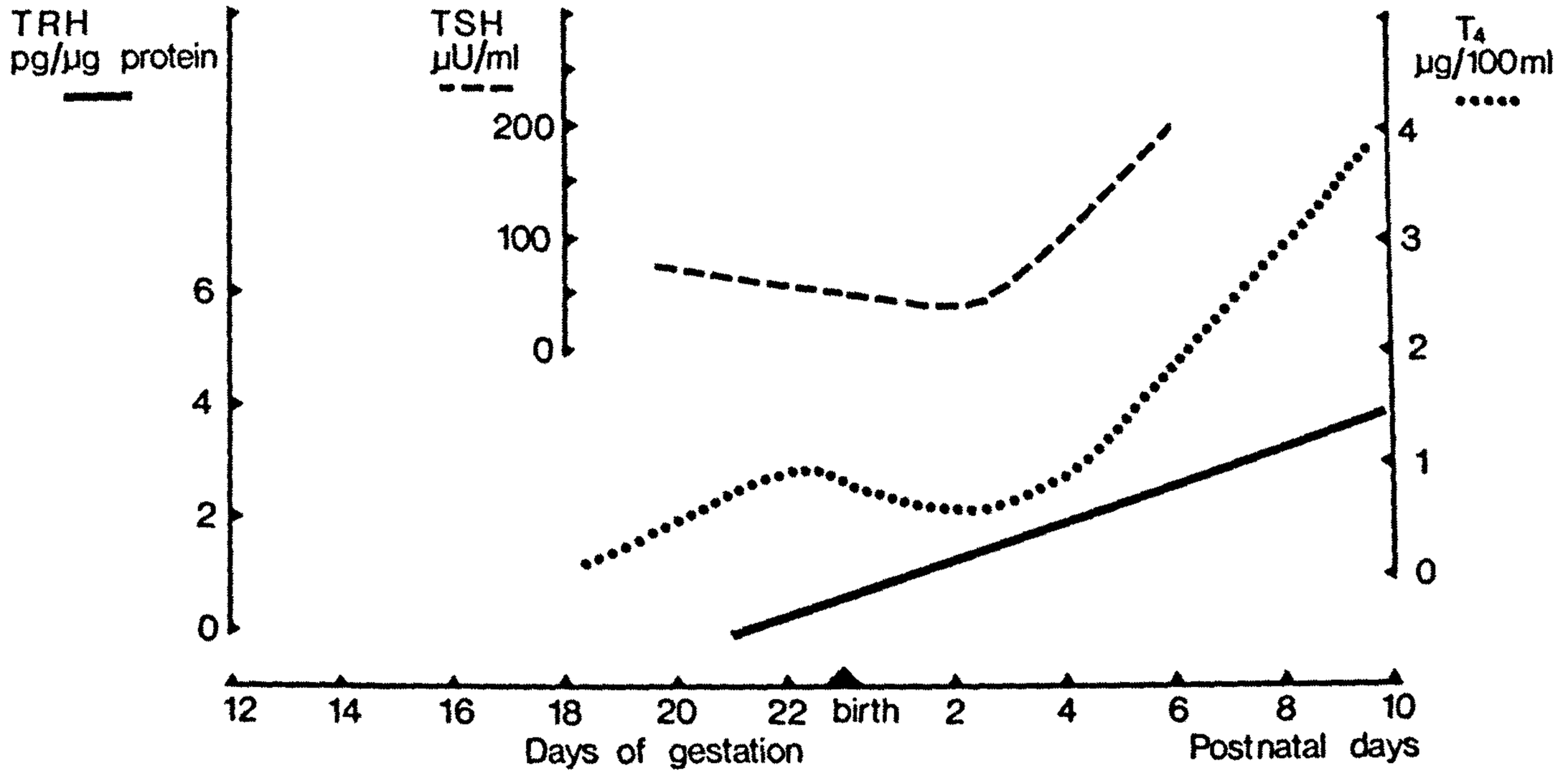

TRH
pg/µg protein
TSH
µU/ml
T4
µg/100ml
200
100
0
6
4
2
0
4
3
2
1
0
12
14
16
18
20
22
birth
2
4
6
8
10
Days of gestation
Postnatal days

Fig. 8

Fig. 9 Effect of LID on 4 hour RAIU.

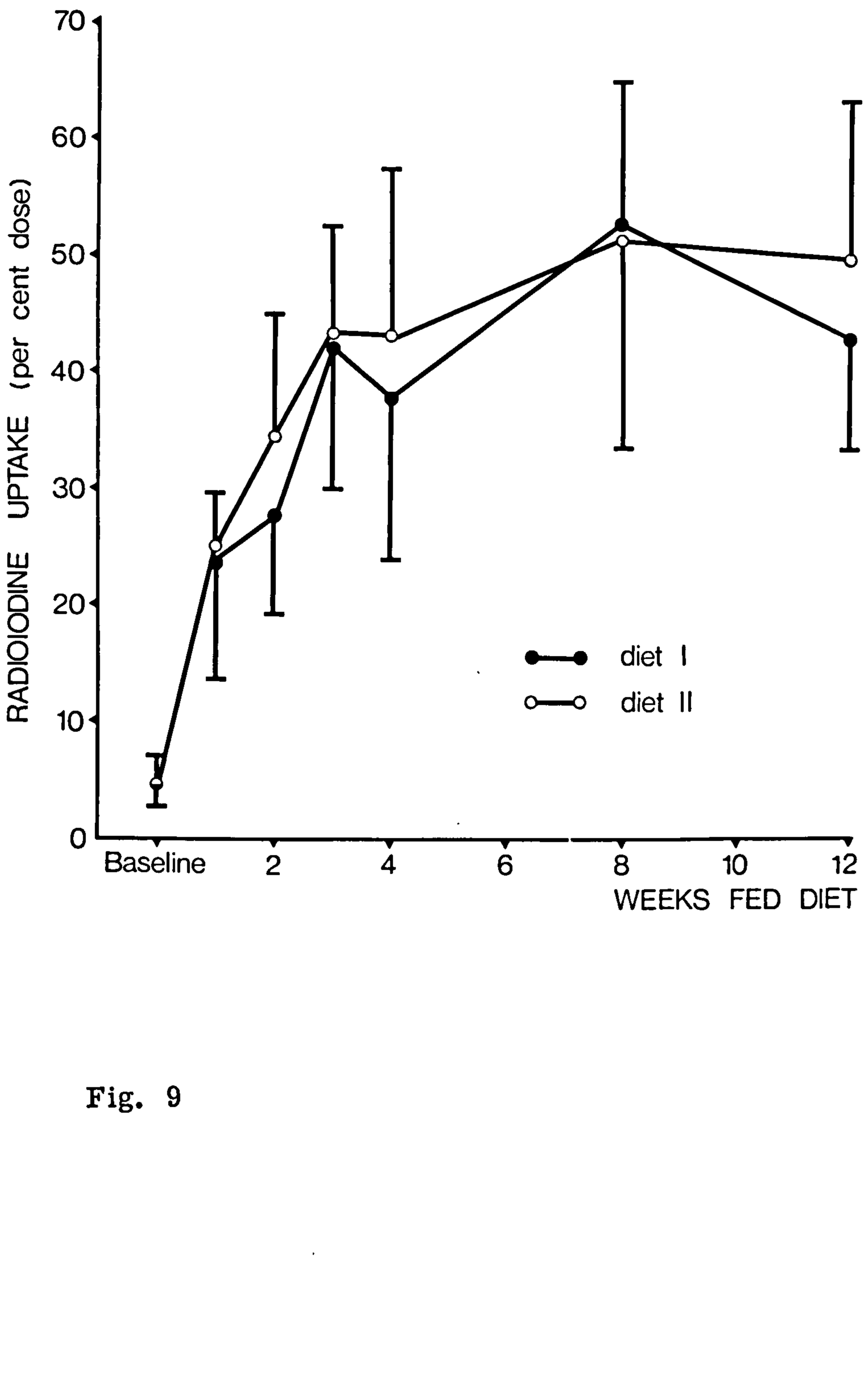

RADIOIODINE UPTAKE (per cent dose)
70
60
50
40
30
20
10
0
Baseline
2
4
6
8
10
12
WEEKS FED DIET
diet I
diet II

Fig. 9

Fig. 10 Effect of LID on relative thyroid weight.

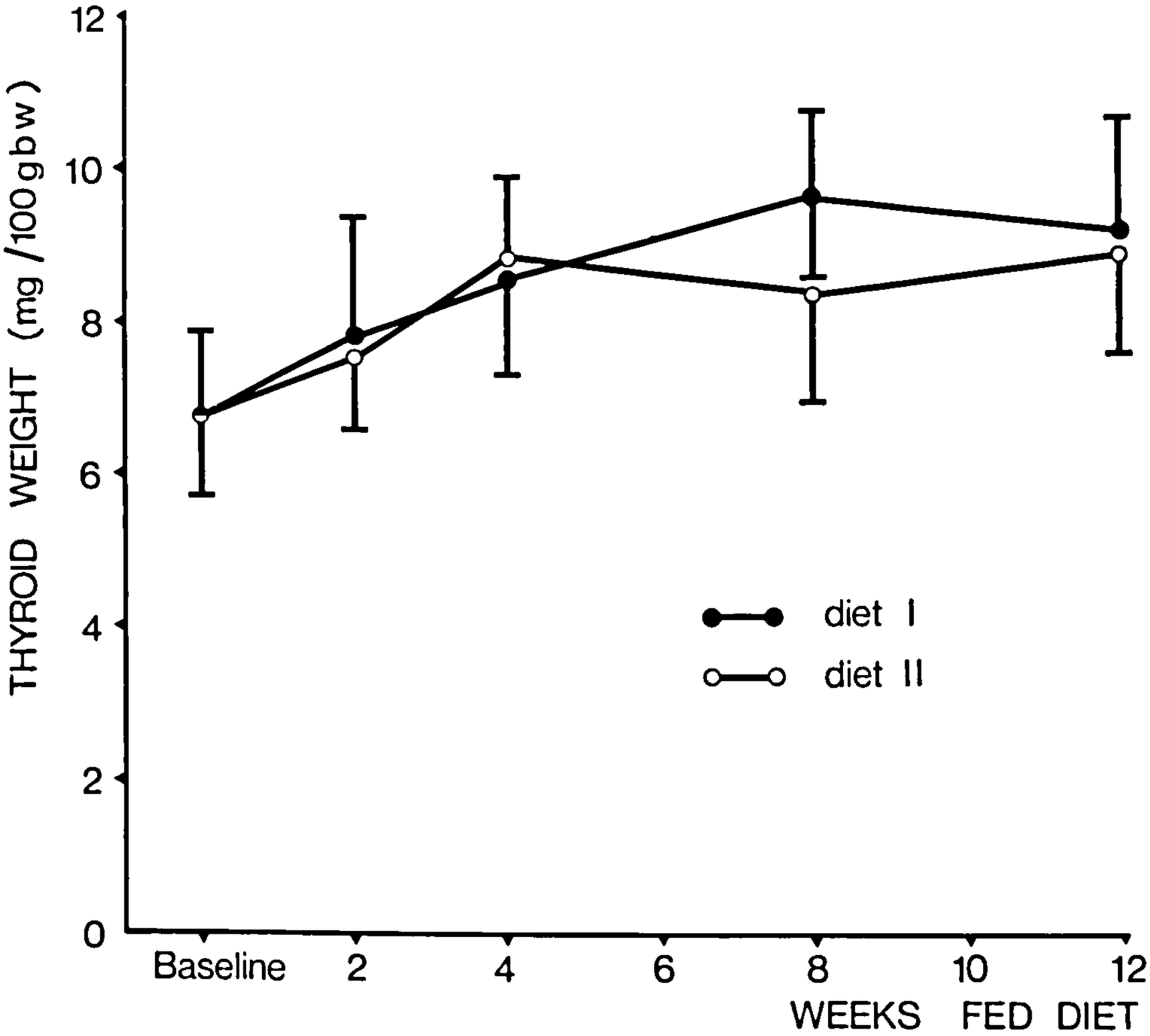

THYROID WEIGHT (mg /100 g b w)
12
10
8
6
4
2
0
Baseline
2
4
6
8
10
12
WEEKS FED DIET
diet I
diet II

Fig. 10

Fig. 11 Effect of LID on serum T_4 concentration.

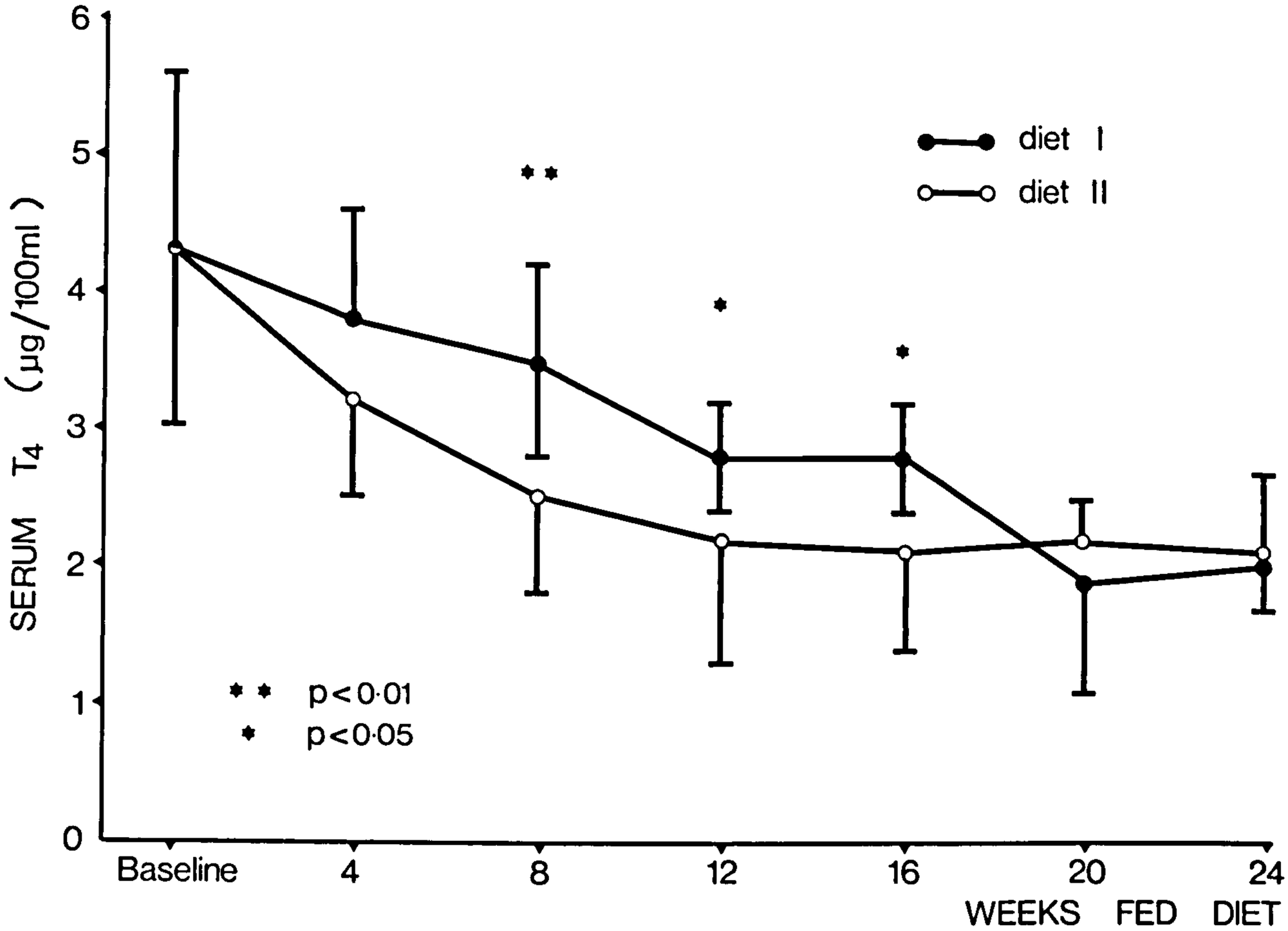

diet I
diet II
SERUM T4 (µg/100ml)
0
1
2
3
4
5
6
Baseline
4
8
12
16
20
24
WEEKS FED DIET
** p<0·01
* p<0·05

Fig. 11

Fig. 12 Comparison of effects of diet I and diet II on serum T_4 concentration.

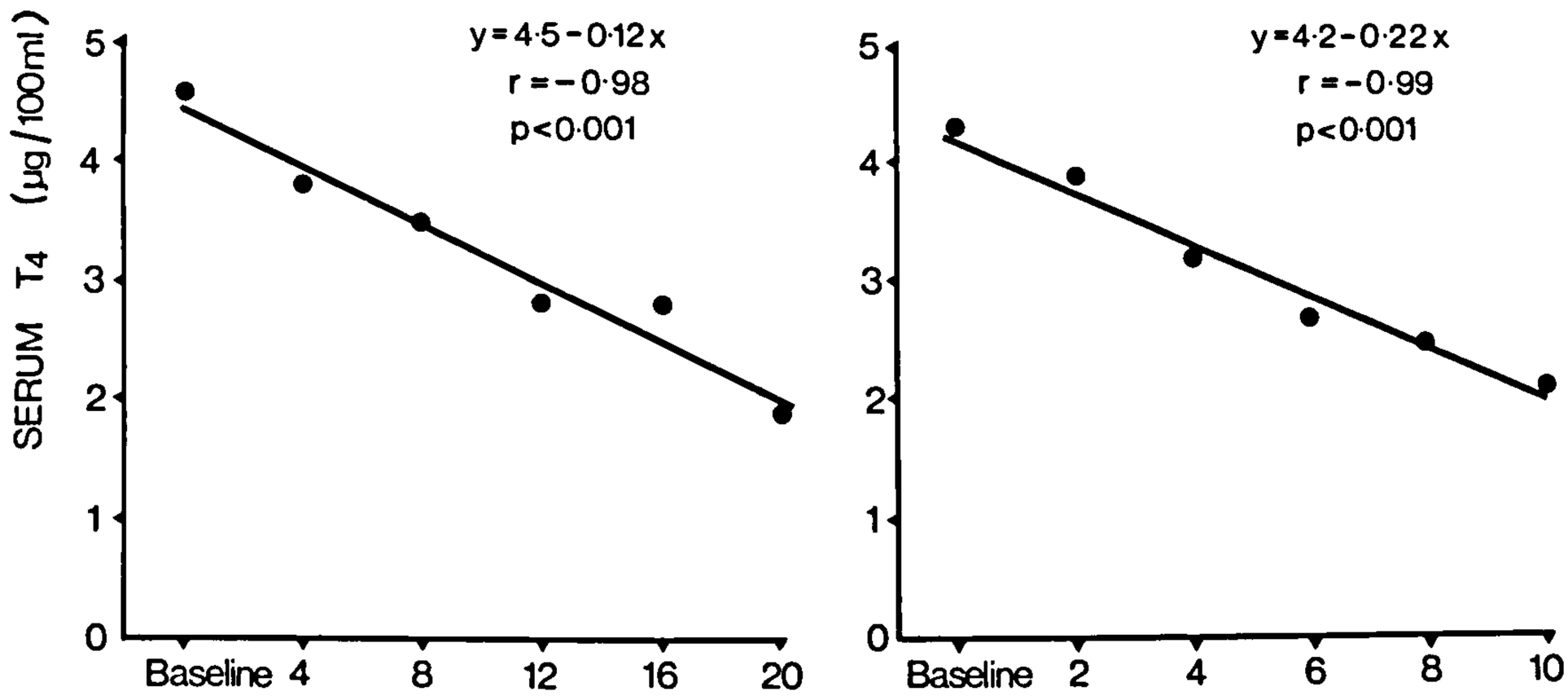

SERUM T4 (µg/100ml)
y=4·5-0·12x
r=-0·98
p<0·001
y=4·2-0·22x
r=-0·99
p<0·001
0
1
2
3
4
5
Baseline 4 8 12 16 20
Baseline 2 4 6 8 10
WEEKS ON DIET I
WEEKS ON DIET II

Fig. 12

Fig. 13 Effect of LID on serum T_3 concentration.


150
120
90
60
30
0
SERUM T3 (ng/100ml)
diet I
diet II
Baseline
4
8
12
16
20
24
WEEKS FED DIET

Fig. 13

Fig. 14 Varying degrees of iodine deficiency.

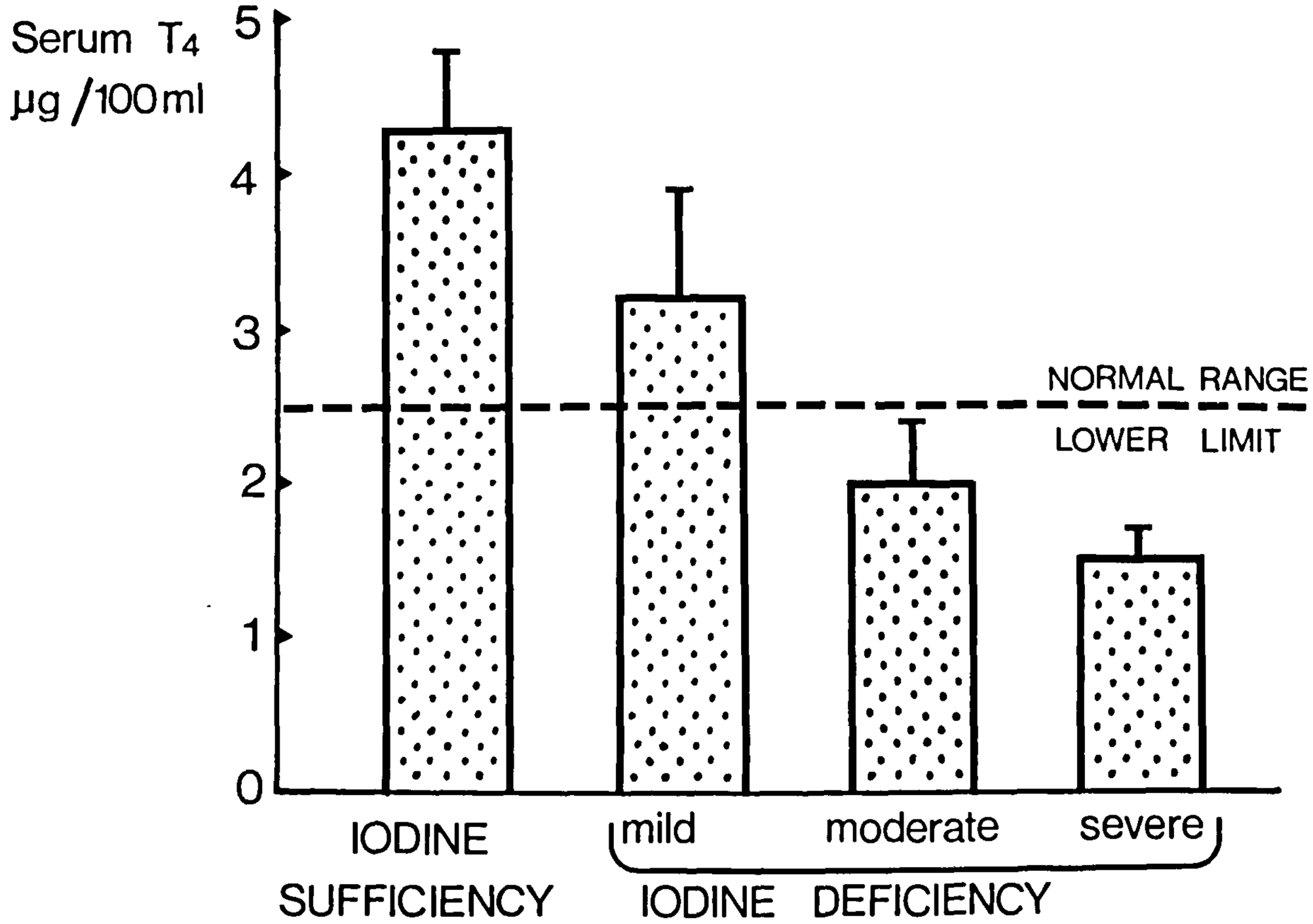

Serum T_4
µg /100ml
5
4
3
2
1
0
NORMAL RANGE
LOWER LIMIT
IODINE
SUFFICIENCY
mild
moderate
severe
IODINE DEFICIENCY

Fig. 14

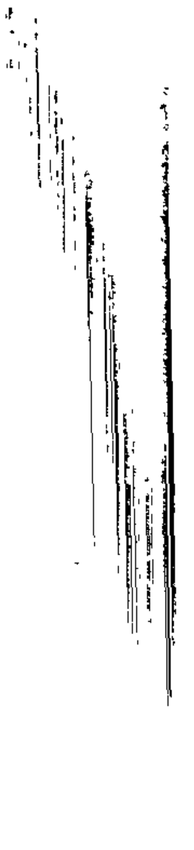


Fig. 15 Postnatal brain development in the rat.
Drawn from data of Donaldson and Hatai (86).

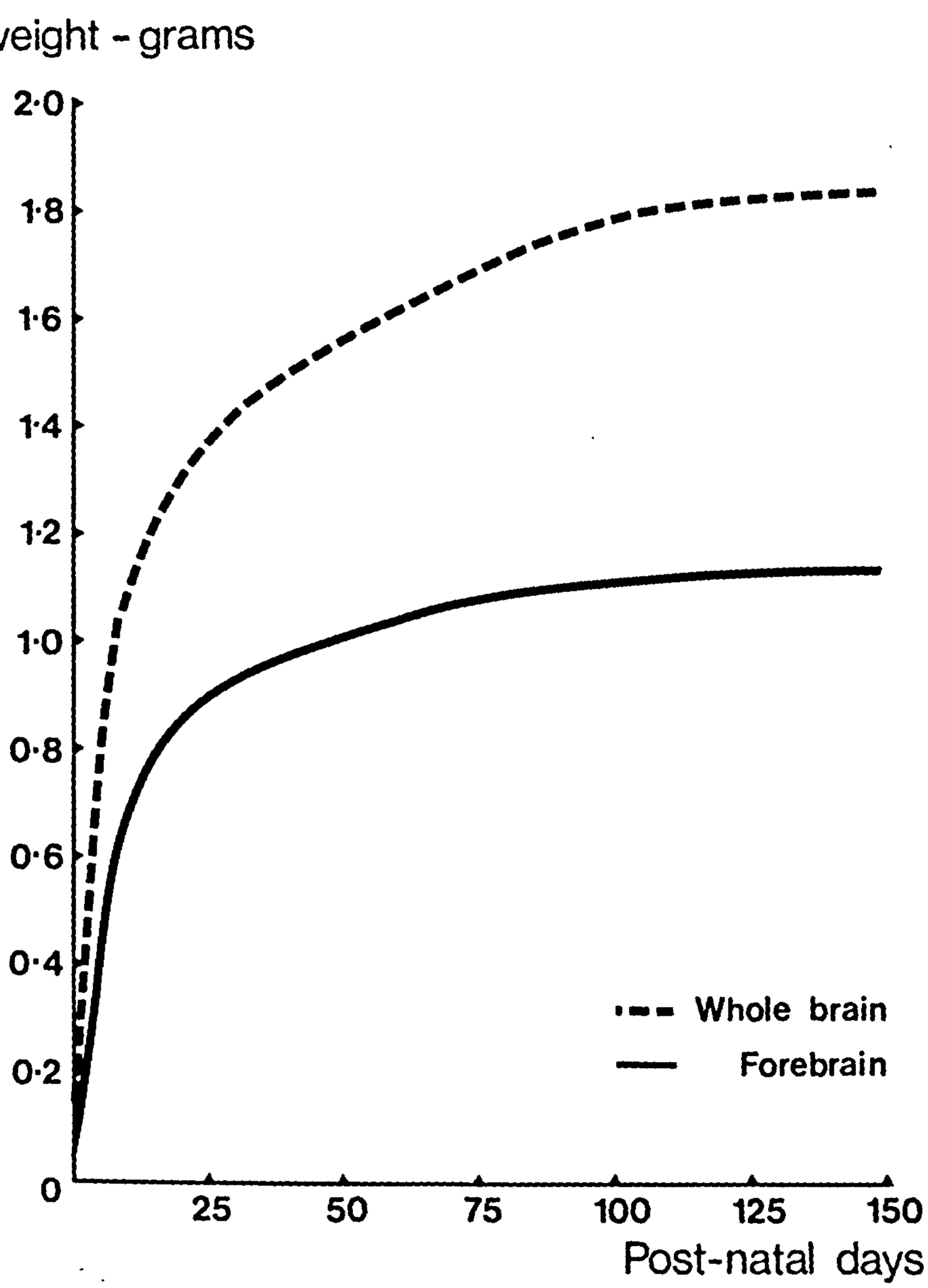

Wet weight - grams
2·0
1·8
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
0
25
50
75
100
125
150
Whole brain
Forebrain
Post-natal days

Fig. 15

Fig. 16 Quantitative changes in DNA and protein during brain growth in the rat. After Winick and Noble (289).

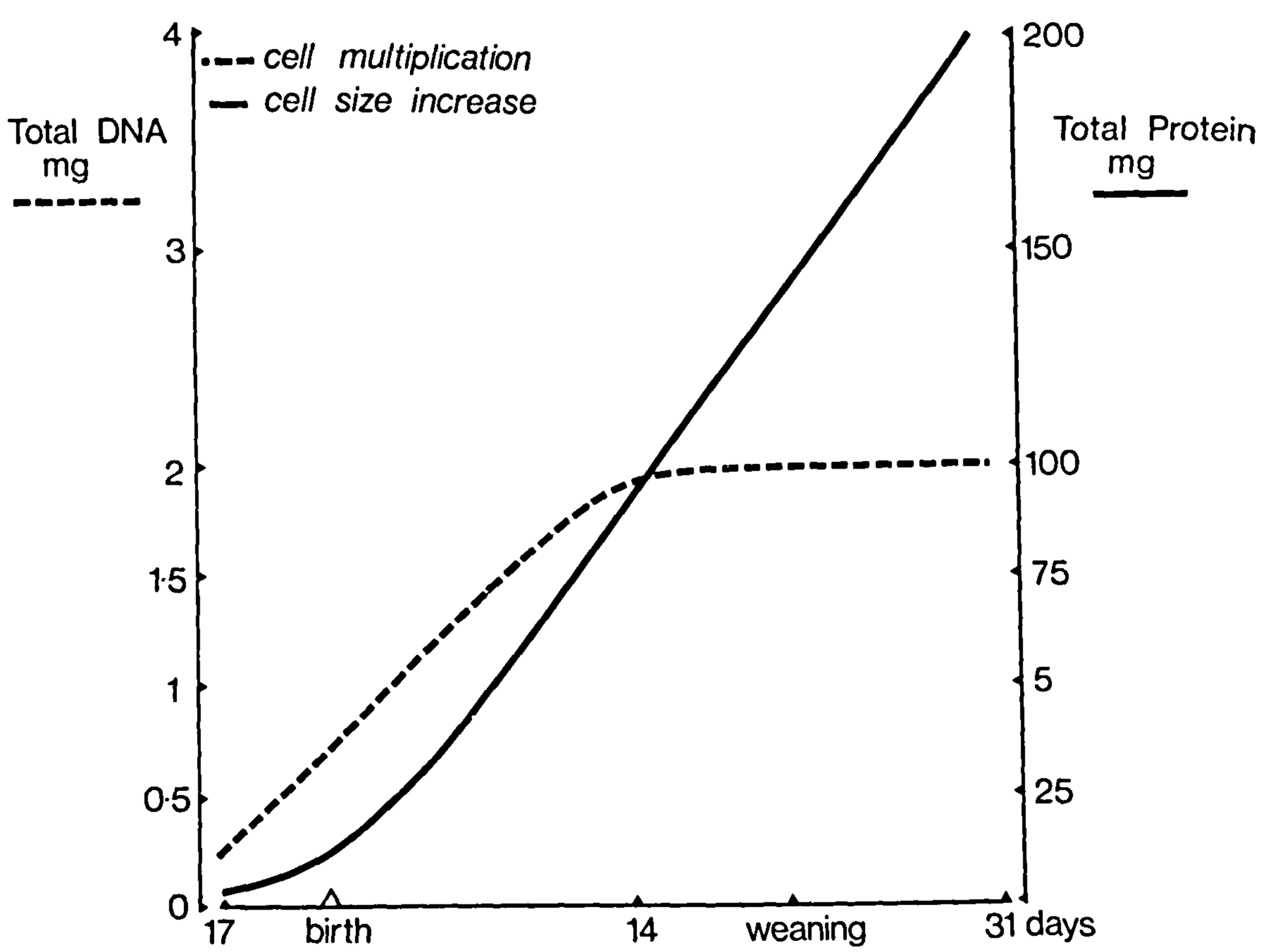

cell multiplication
cell size increase
Total DNA mg
Total Protein mg
4
3
2
1·5
1
0·5
0
200
150
100
75
5
25
17
birth
14
weaning
31 days

Fig. 16

Fig. 17 Quantitative changes in DNA during forebrain growth in man. After Dobbing and Sands (79).

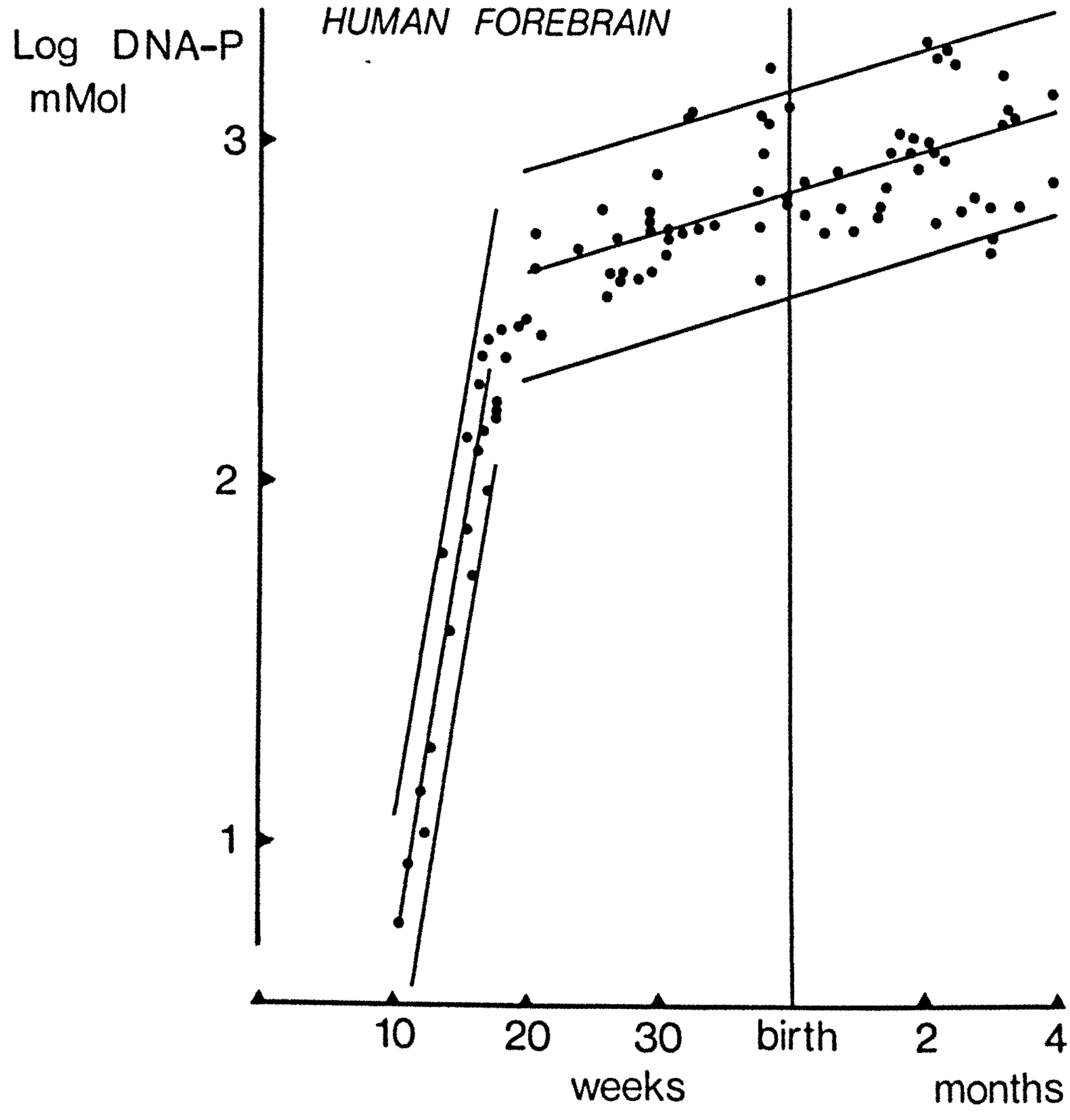
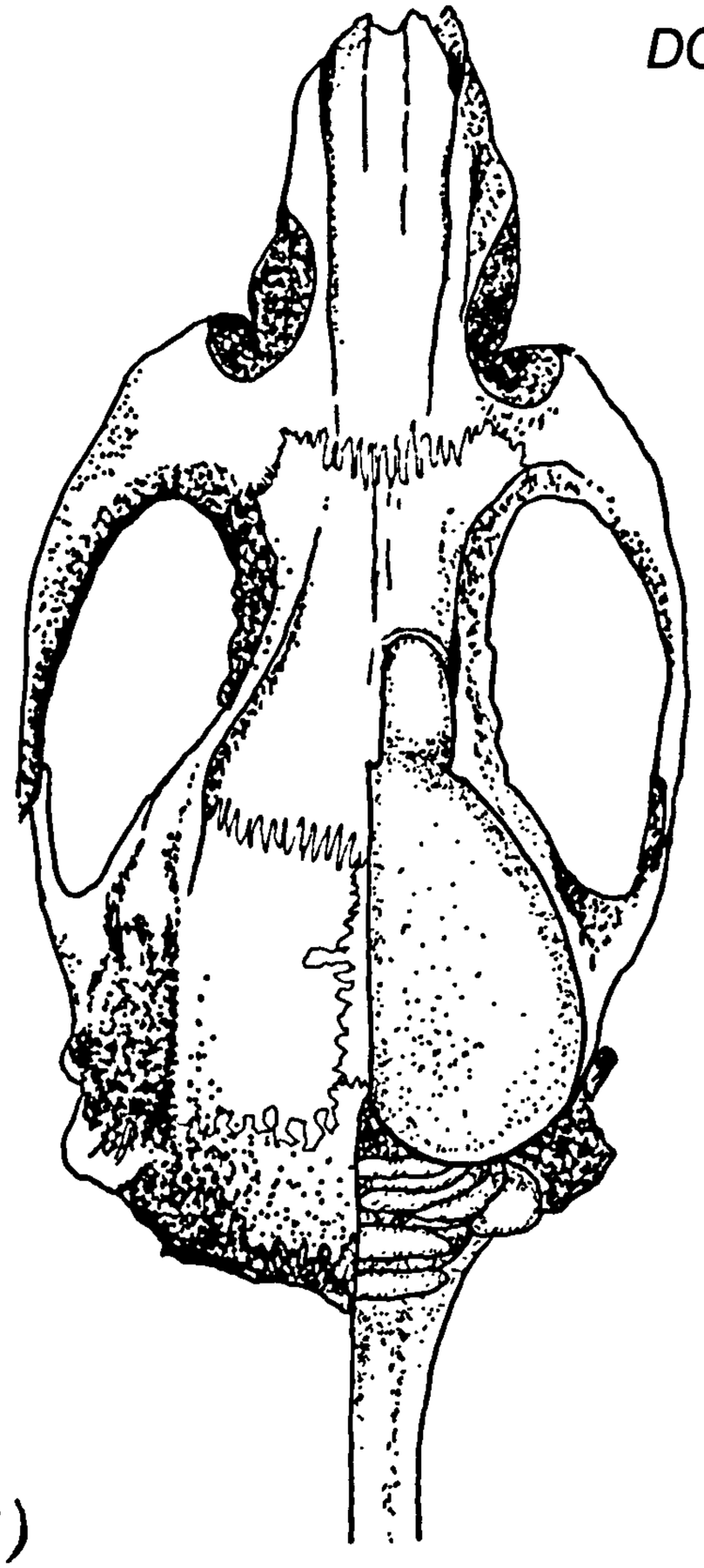

HUMAN FOREBRAIN
Log DNA-P
mMol
3
2
1
10
20
30
birth
2
4
weeks
months

Fig. 17

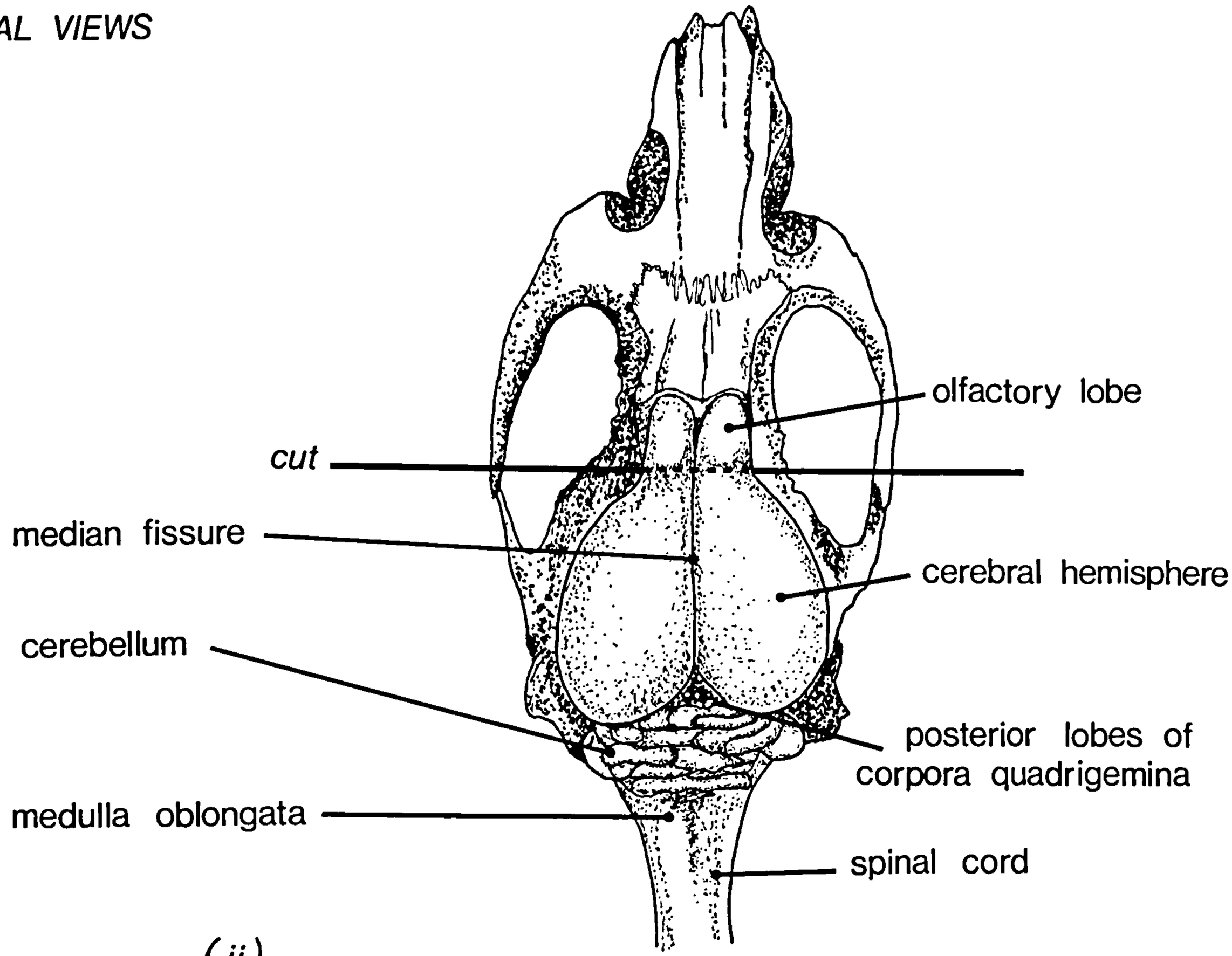
Fig. 18 Method of dissection of rat forebrain.
Steps (i) and (ii).

DISSECTON OF RAT FOREBRAIN

DORSAL VIEWS



(i)

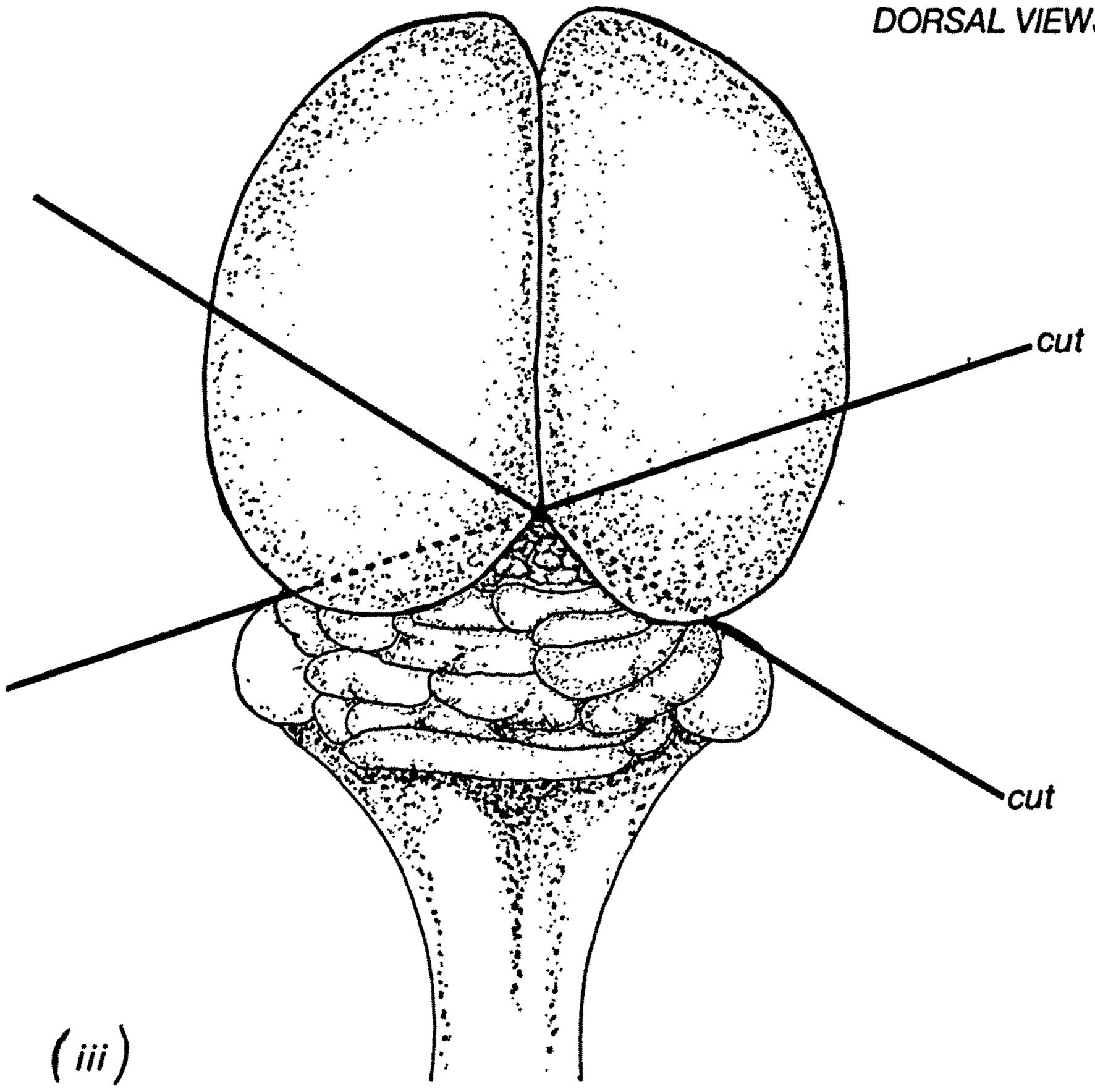


(ii)

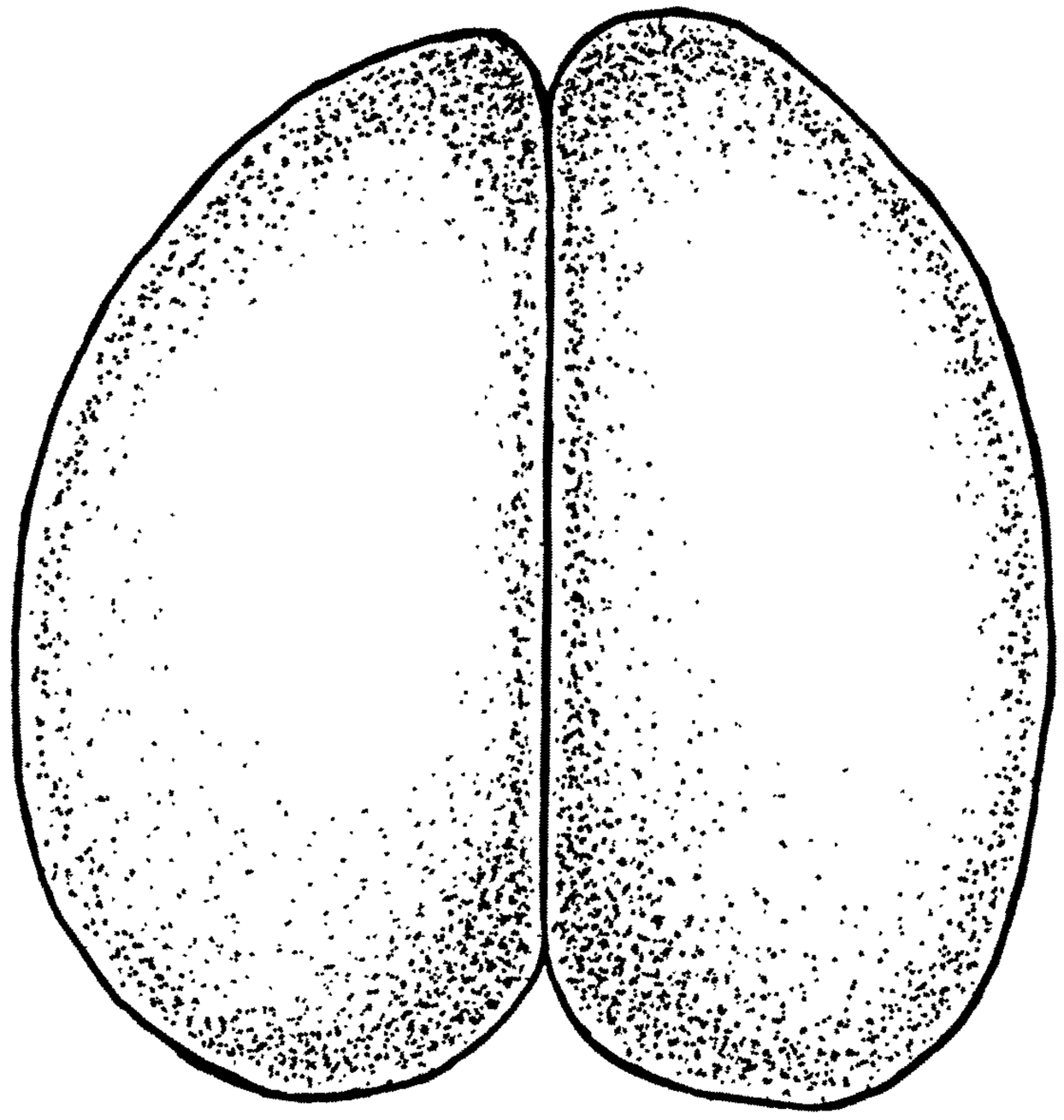
Fig. 18 Method of dissection of rat forebrain.
Steps (iii) and (iv).

DISSECTION OF RAT FOREBRAIN

DORSAL VIEWS



(iii)



(iv)

Fig. 19 Biochemical analysis of rat forebrain.

RAT FOREBRAIN

COLD ACID EXTRACTION

(homogenised in 7 ml 6% TCA, spun at 18,000 g for 40 min.)

ACID-SOLUBLE FRACTION

(small molecules e.g. free nucleotides, potential source of interference with diphenylamine reaction for DNA)

TWO HOT ACID EXTRACTIONS

(pellet suspended in 2 ml 1M $HClO_4$ at $70^{o}C$ for 15 min., spun at 18,000 g for 40 min.)

Acid extract containing hydrolysis products of DNA

DIPHENYLAMINE REACTION

TOTAL DNA

Residue of protein

ALKALINE HYDROLYSIS

(2 ml 1N NaOH)

FOLIN PHENOL REACTION

TOTAL PROTEIN

Fig. 19

Fig. 20 Typical standard curve for DNA assay.

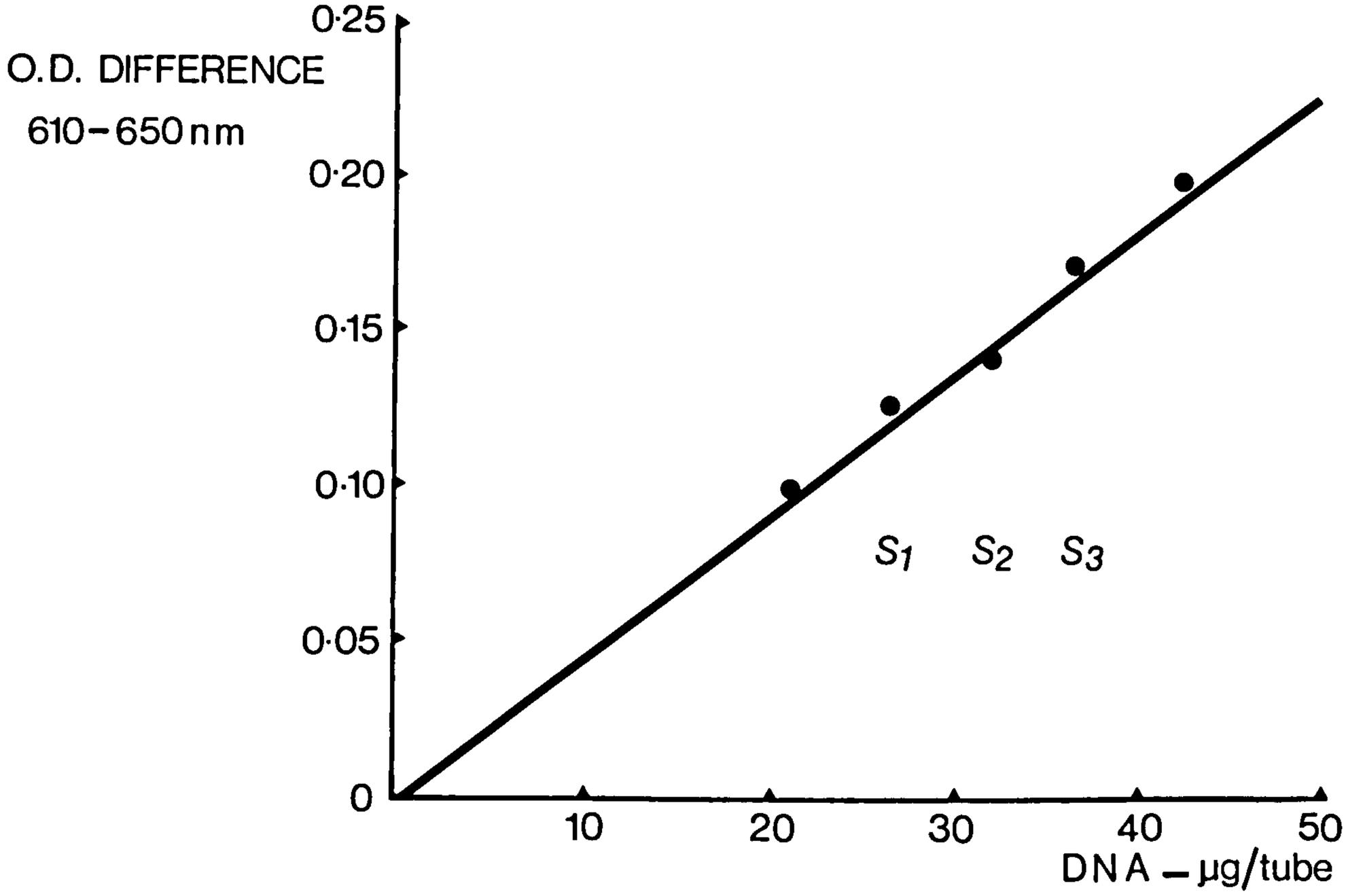

O.D. DIFFERENCE
610–650 nm
0·25
0·20
0·15
0·10
0·05
0
10
20
30
40
50
DNA – µg/tube
S1
S2
S3

Fig. 20

Fig. 21 Typical standard curve for protein assay.

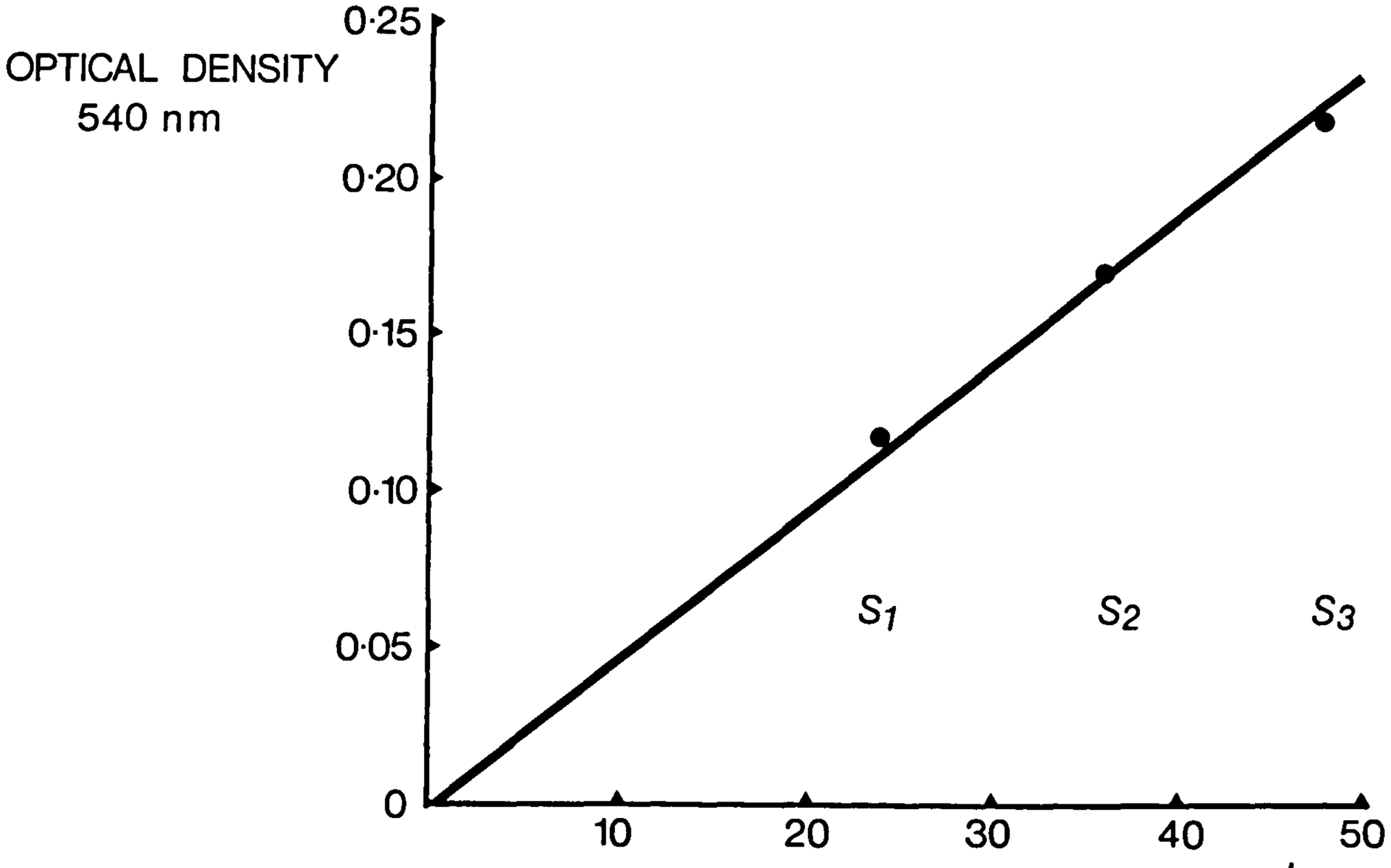

OPTICAL DENSITY
540 nm
0·25
0·20
0·15
0·10
0·05
0
10
20
30
40
50
S1
S2
S3
PROTEIN – µg/tube

Fig. 21

Fig. 22 Quantitative changes in DNA and protein during postnatal forebrain growth.

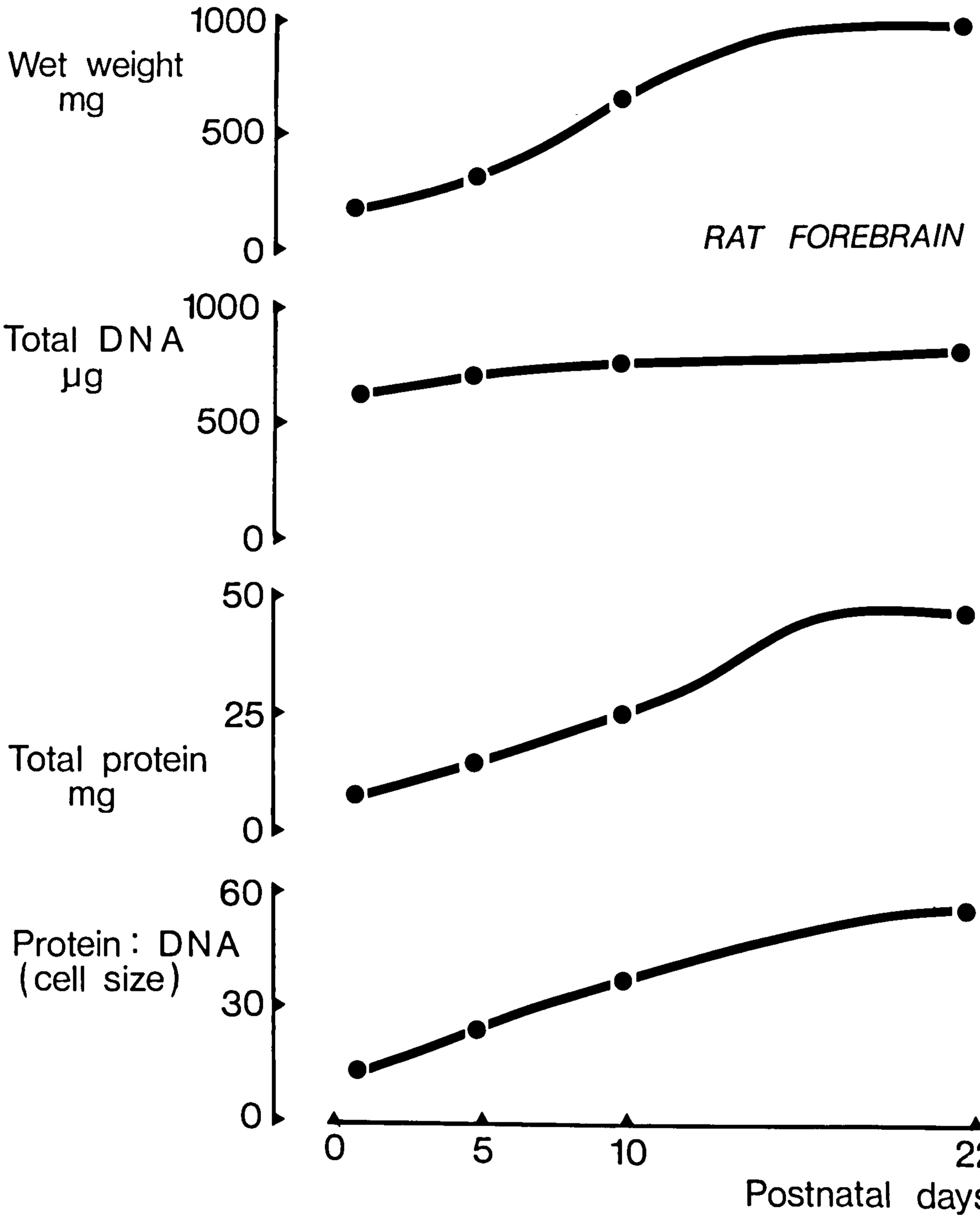

Wet weight mg
1000
500
0
RAT FOREBRAIN
Total DNA µg
1000
500
0
Total protein mg
50
25
0
Protein : DNA (cell size)
60
30
0
0
5
10
22
Postnatal days

Fig. 22

Fig. 23 Design of experiments 1-4.

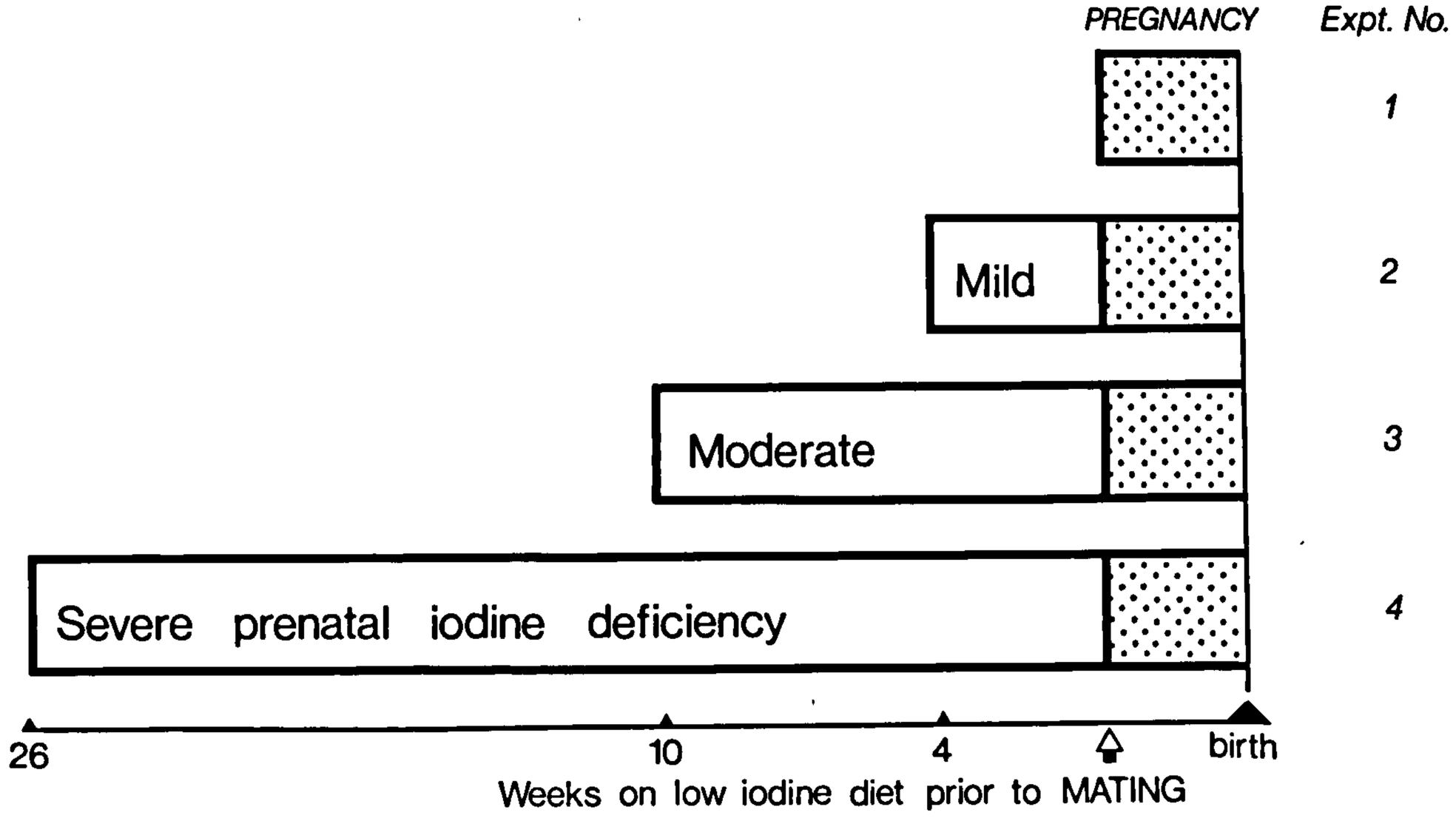

PREGNANCY
Expt. No.
1
Mild
2
Moderate
3
Severe prenatal iodine deficiency
4
26
10
4
birth
Weeks on low iodine diet prior to MATING

Fig. 23

Fig. 24 Growth of rats on LID for 4 weeks.

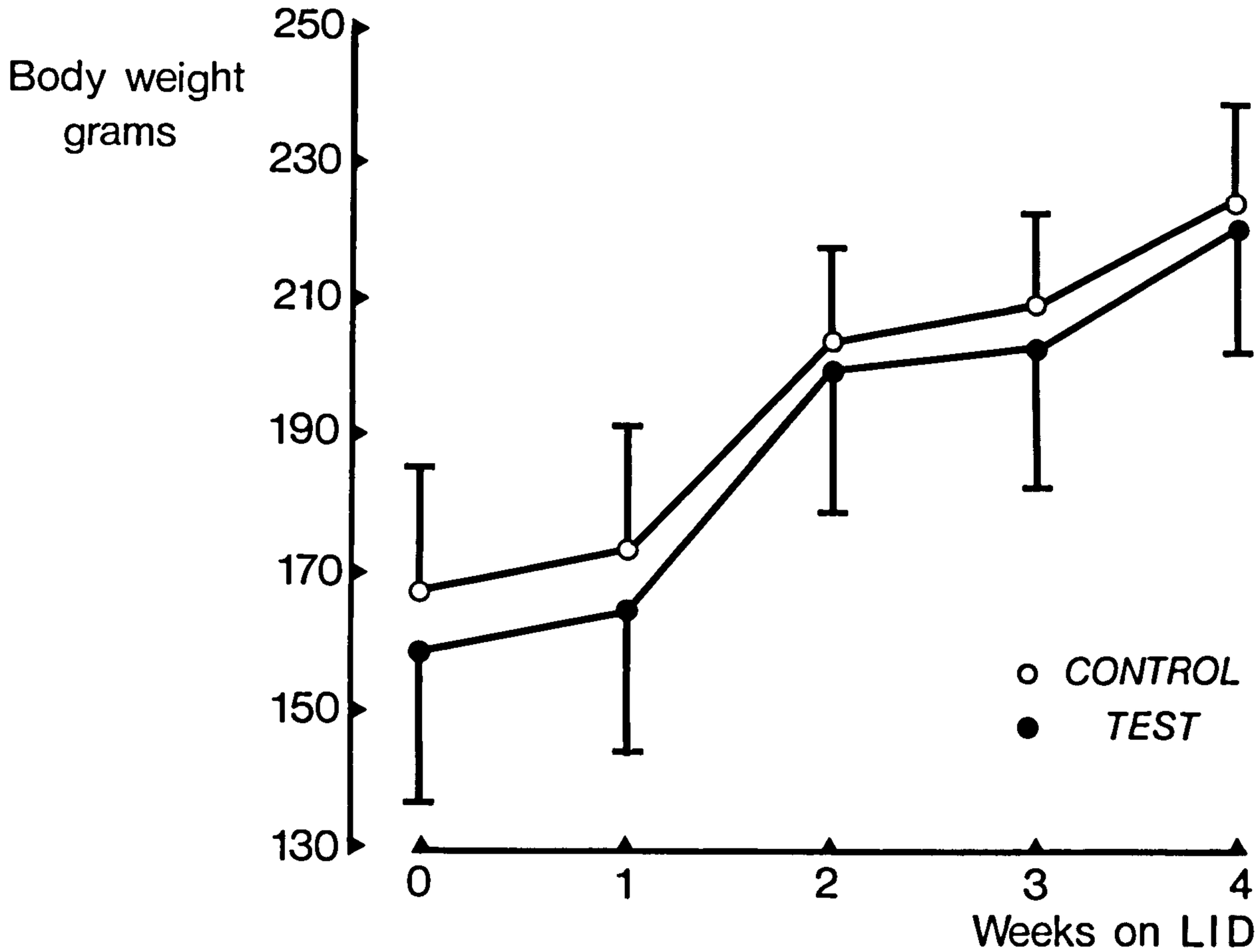

Body weight
grams
250
230
210
190
170
150
130
0
1
2
3
4
Weeks on LID
CONTROL
TEST

Fig. 24

Fig. 25 Changes in serum T_4 and T_3 concentrations of animals mated after 4 weeks on LID.

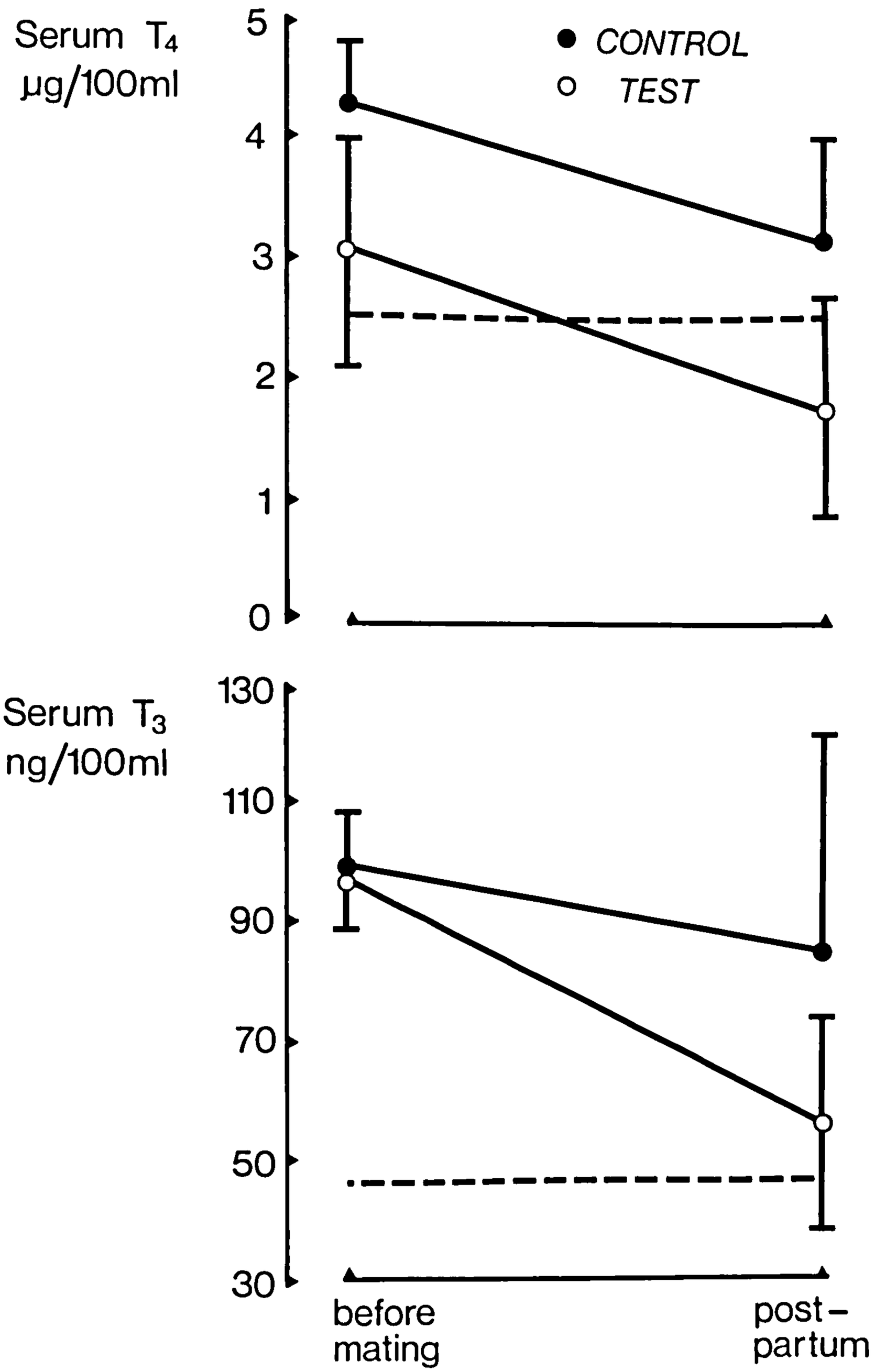

Serum T_4
µg/100ml
5
4
3
2
1
0
CONTROL
TEST
Serum T_3
ng/100ml
130
110
90
70
50
30
before
mating
post–
partum

Fig. 25

Fig. 26 Comparison of serum T_4 concentrations in test and control animals after 8 weeks on LID. Broken line denotes the lower limit of the normal range. The means $\pm$ 1 S.D. are shown for the animals selected for mating after 10 weeks.

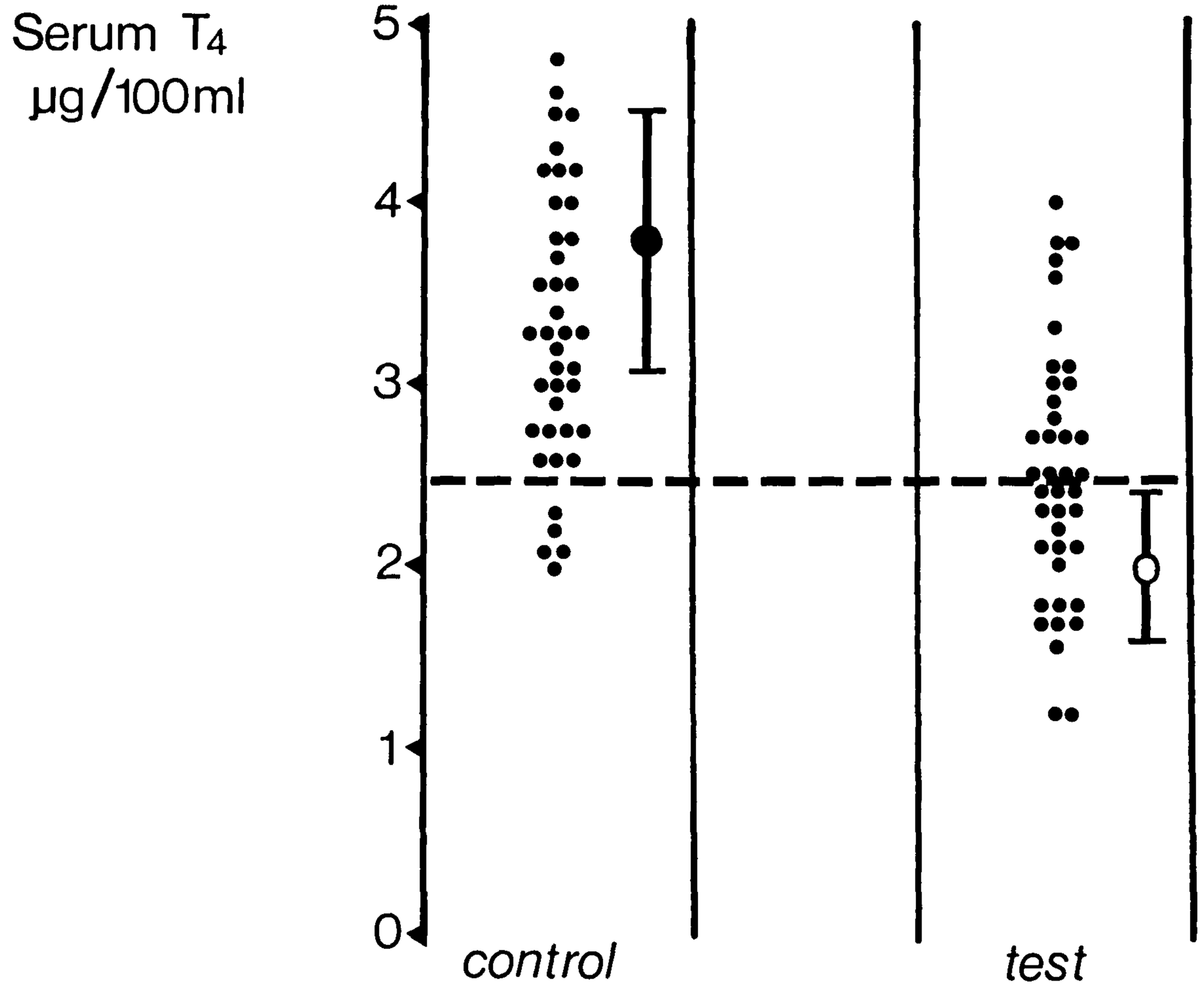


Fig. 26

Fig. 27 Changes in serum T_4 and T_3 concentrations of animals mated after 10 weeks on LID.

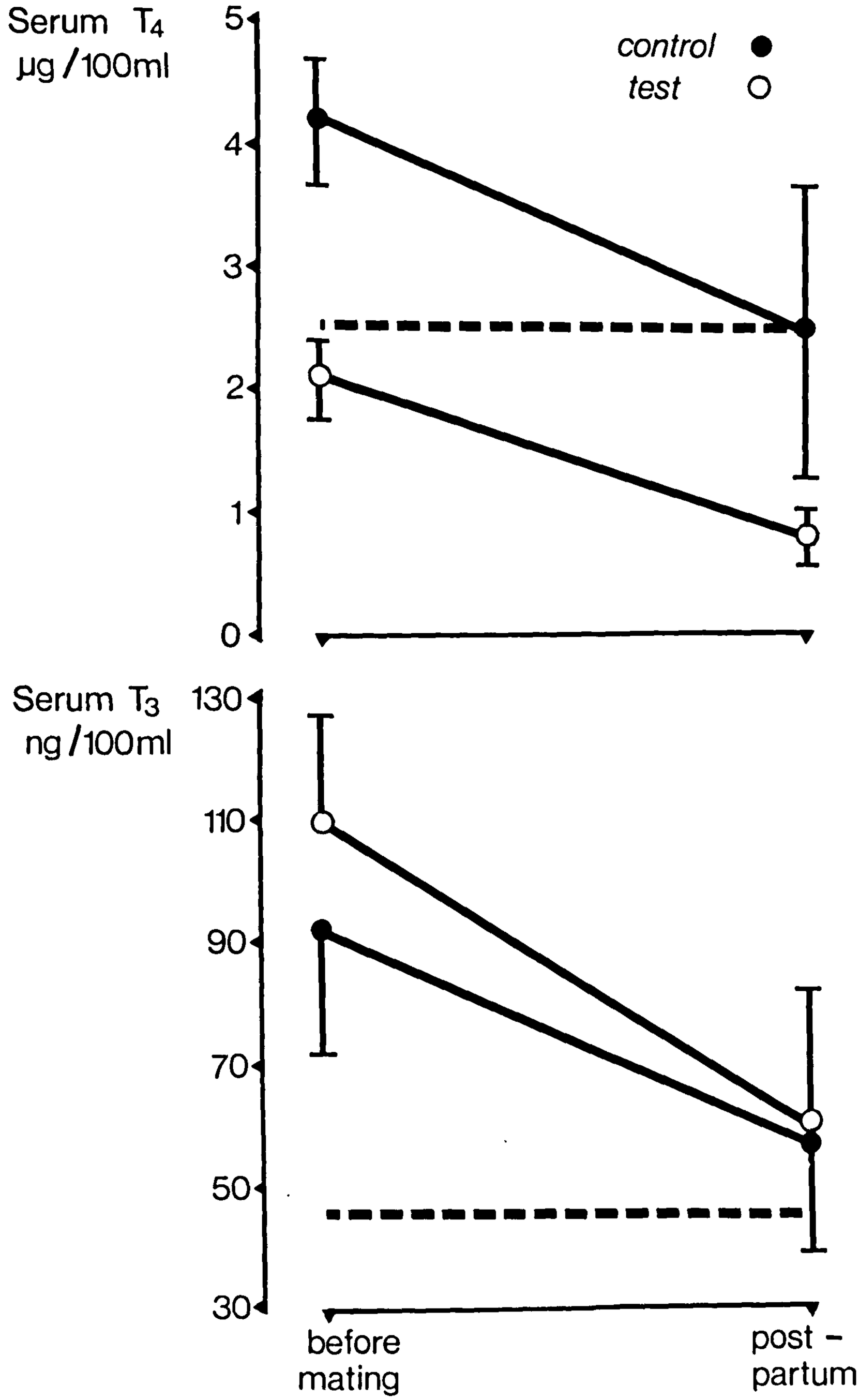

Serum T_4
µg/100ml
5
4
3
2
1
0
control
test
Serum T_3
ng/100ml
130
110
90
70
50
30
before mating
post - partum

Fig. 27

Fig. 28 Changes in serum T_4 and T_3 concentrations of animals mated after 6 months on LID.

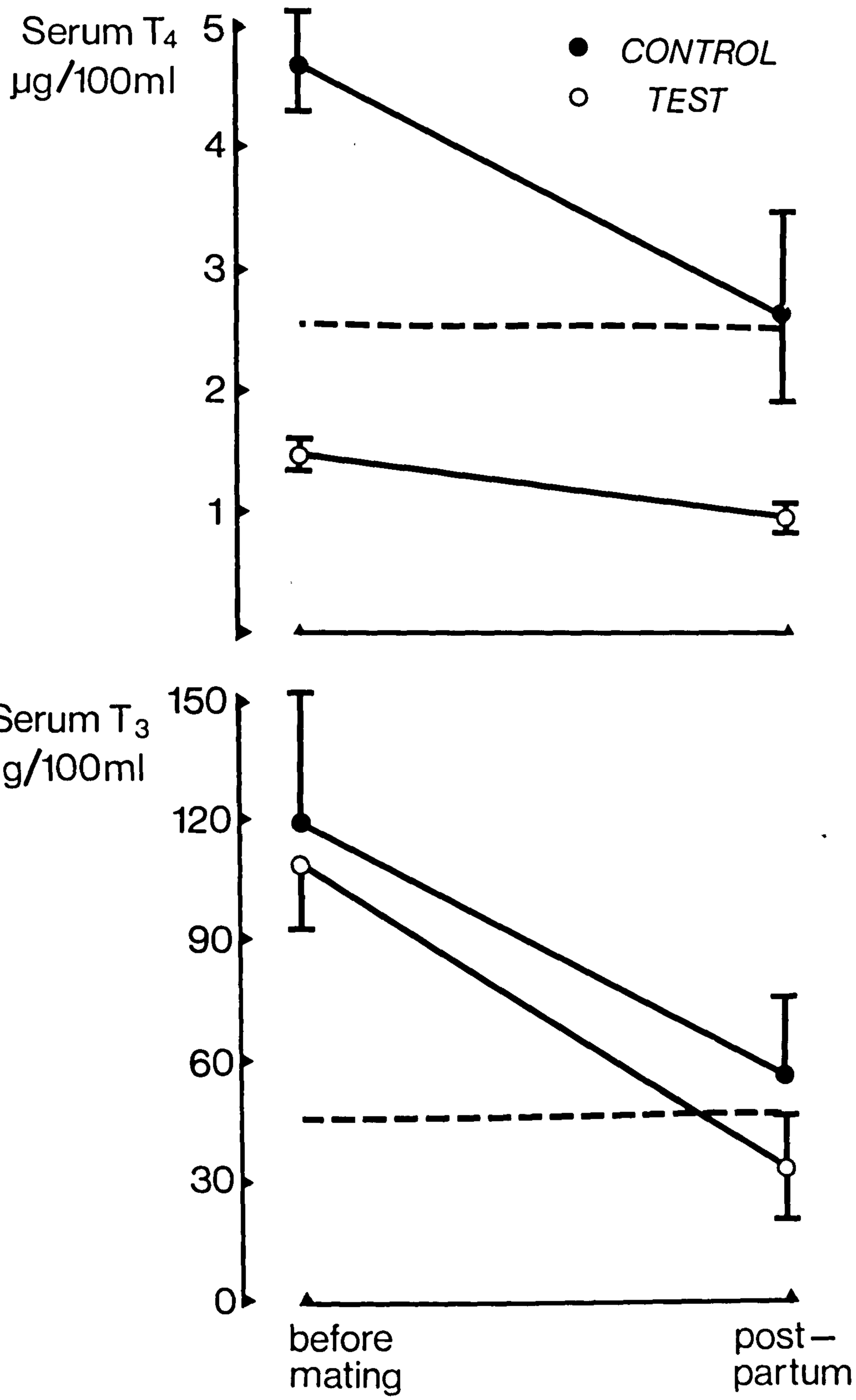

Serum T_4
µg/100ml
5
4
3
2
1
CONTROL
TEST
Serum T_3
ng/100ml
150
120
90
60
30
0
before
mating
post–
partum

Fig. 28

Fig. 29 Comparison of growth rate in test and control pups during first 3 postnatal weeks.

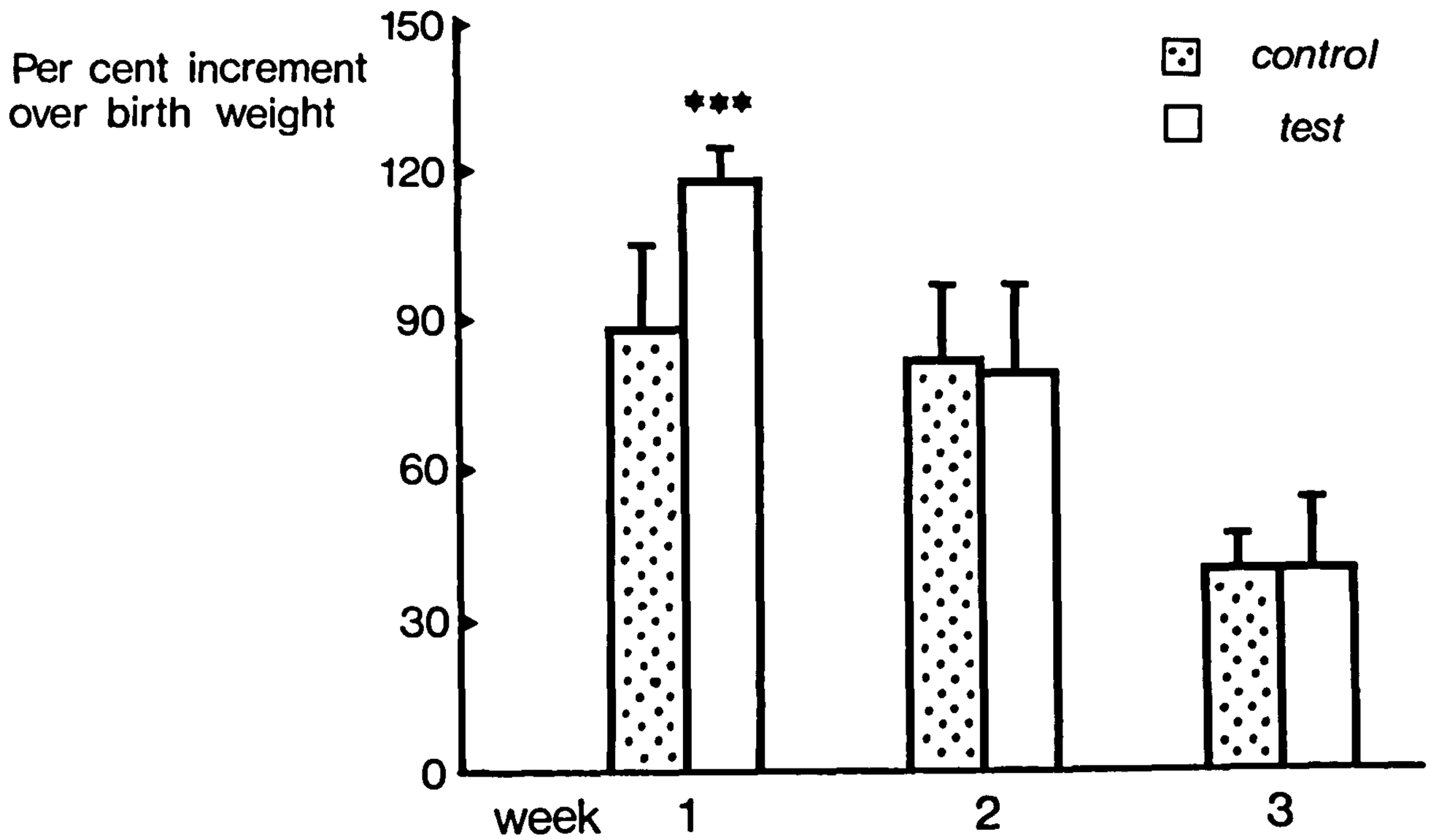

Per cent increment
over birth weight
150
120
90
60
30
0
***
control
test
week
1
2
3

Fig. 29

Fig. 30 Changes in serum T_4 and T_3 concentrations during pregnancy and lactation in Experiment 5.

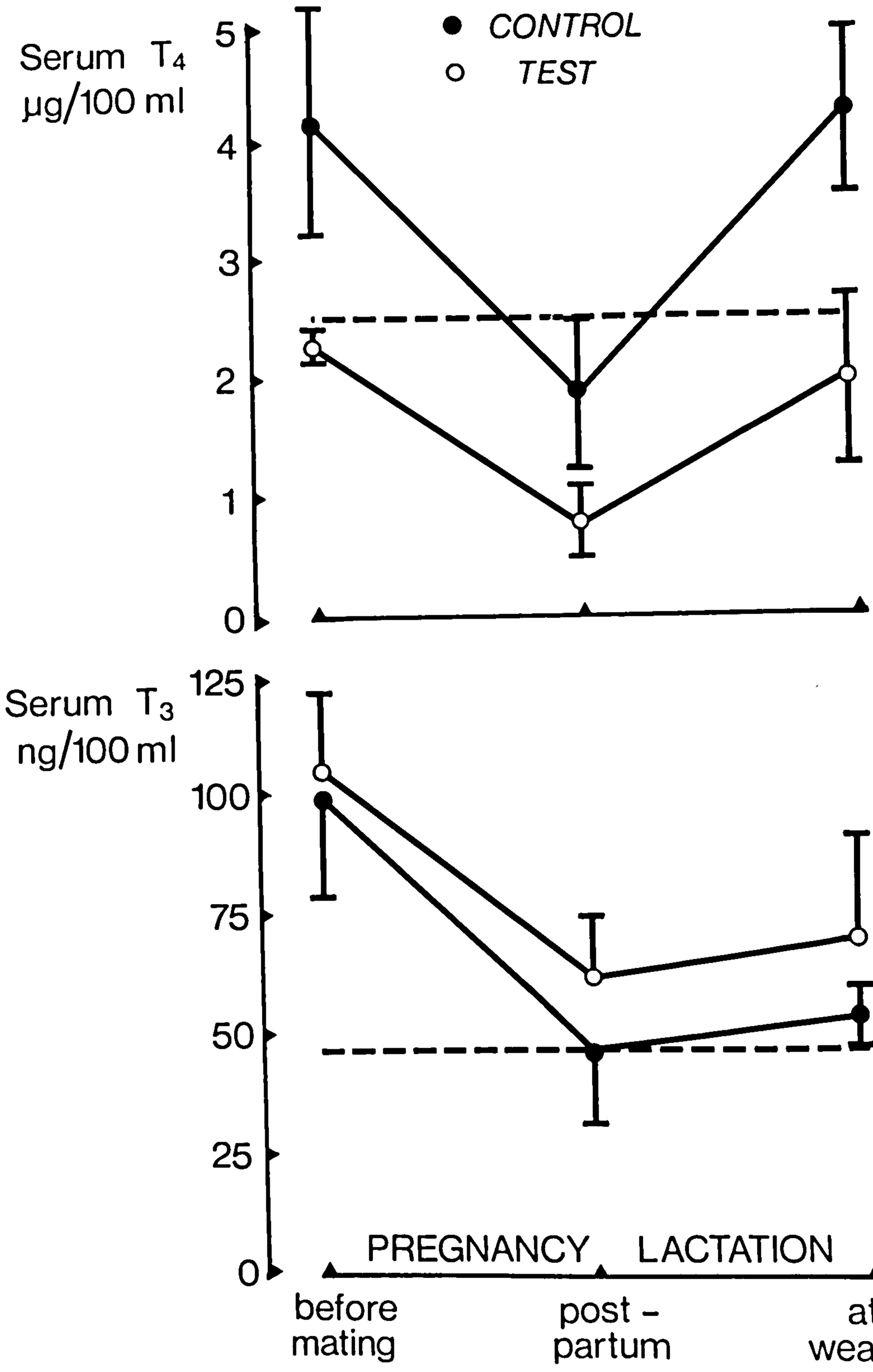

Serum T_4
µg/100 ml
5
4
3
2
1
0
CONTROL
TEST
Serum T_3
ng/100 ml
125
100
75
50
25
0
PREGNANCY
LACTATION
before mating
post - partum
at weaning

Fig. 30

Fig. 31 Changes in thyroid hormone concentrations during pregnancy in Experiments 2, 3 and 4.

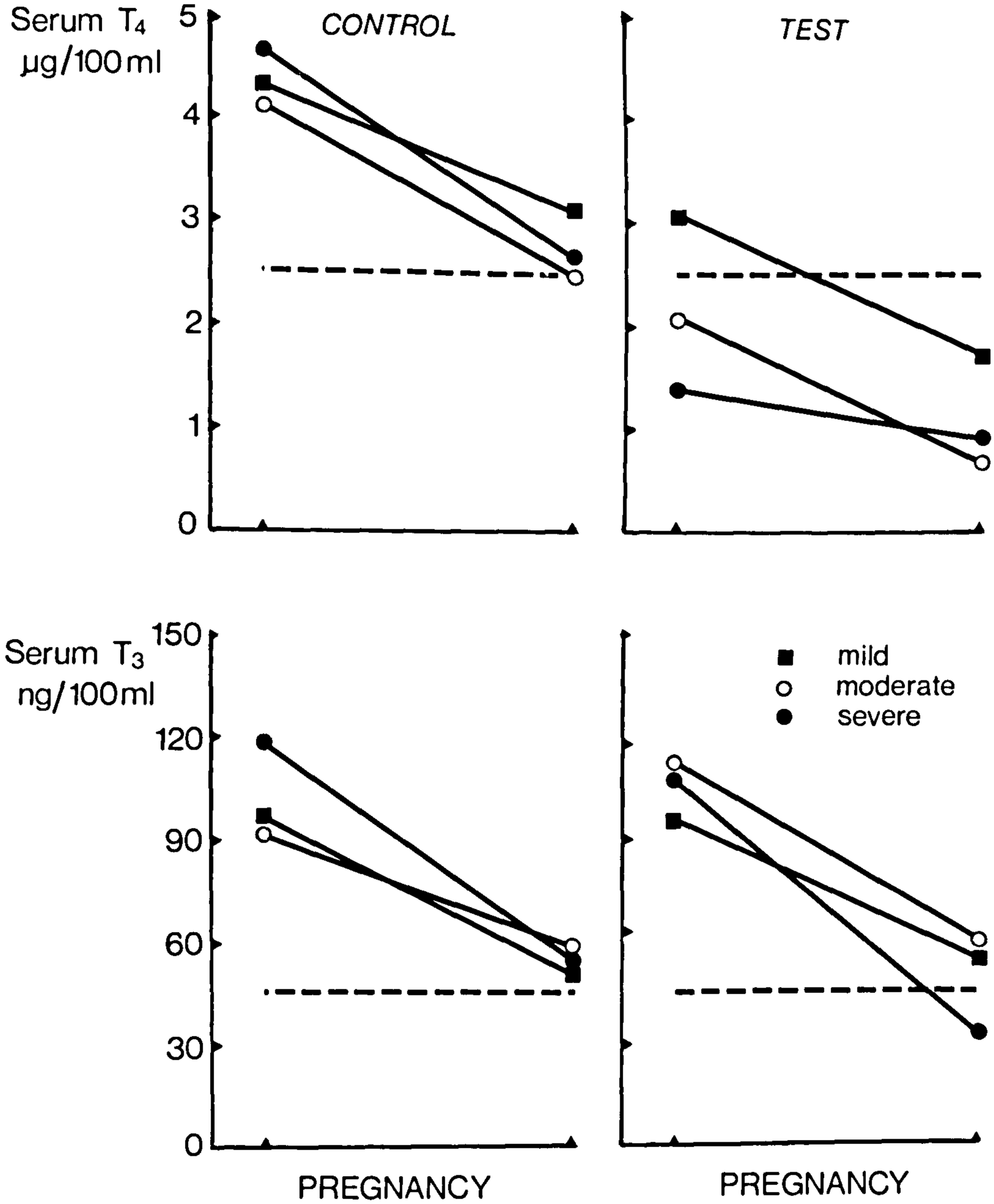

Serum T_4 µg/100ml
5
4
3
2
1
0
CONTROL
TEST
Serum T_3 ng/100ml
150
120
90
60
30
0
mild
moderate
severe
PREGNANCY
PREGNANCY

Fig. 31

Fig. 32 Changes in serum T_4 concentration due to pregnancy in Experiment 6.

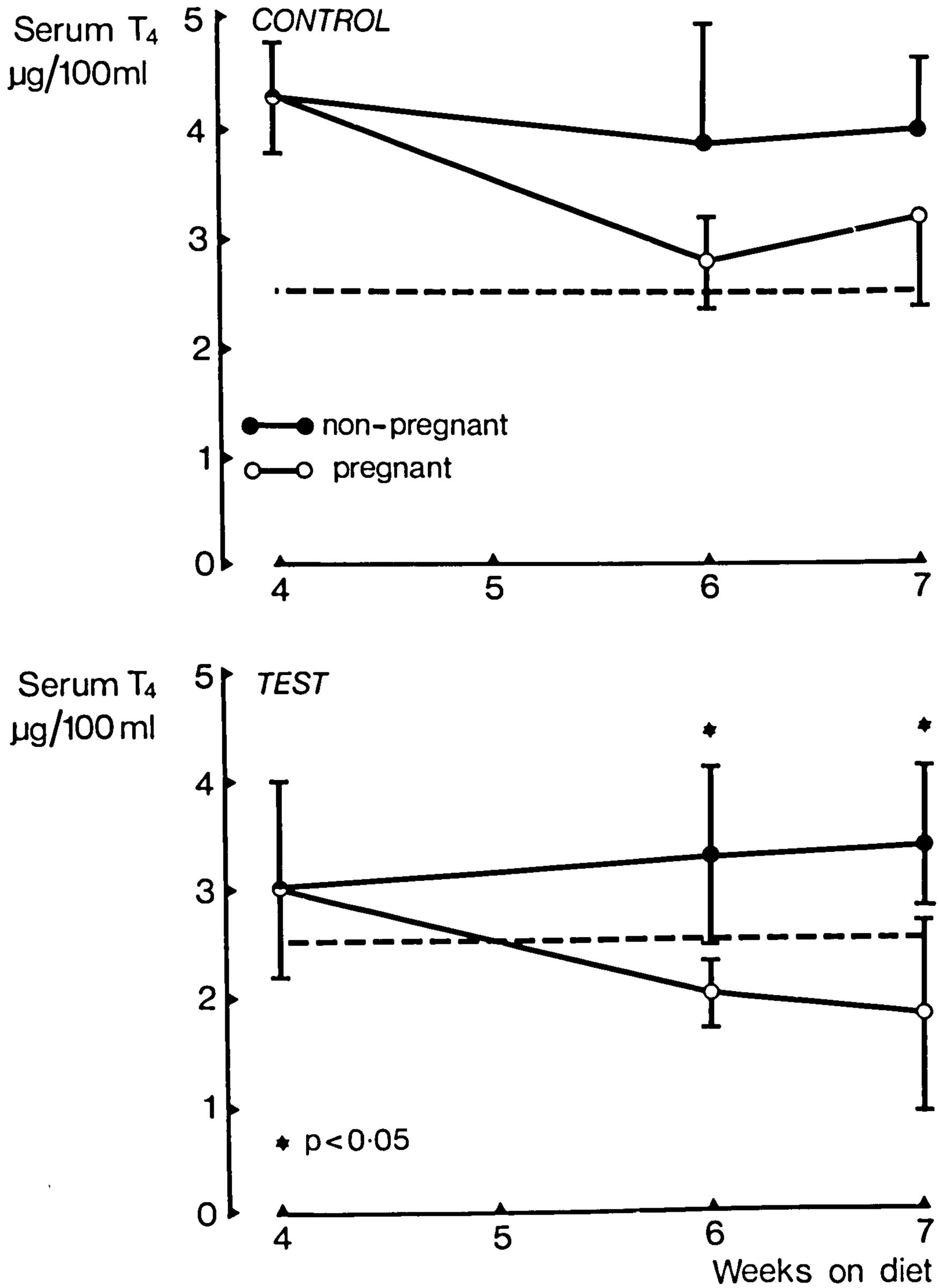

Serum T_4 µg/100ml
CONTROL
5
4
3
2
1
0
non-pregnant
pregnant
4
5
6
7
Serum T_4 µg/100 ml
TEST
* p<0·05
Weeks on diet

Fig. 32

Fig. 33 Changes in serum T_3 concentration due to pregnancy in Experiment 6.

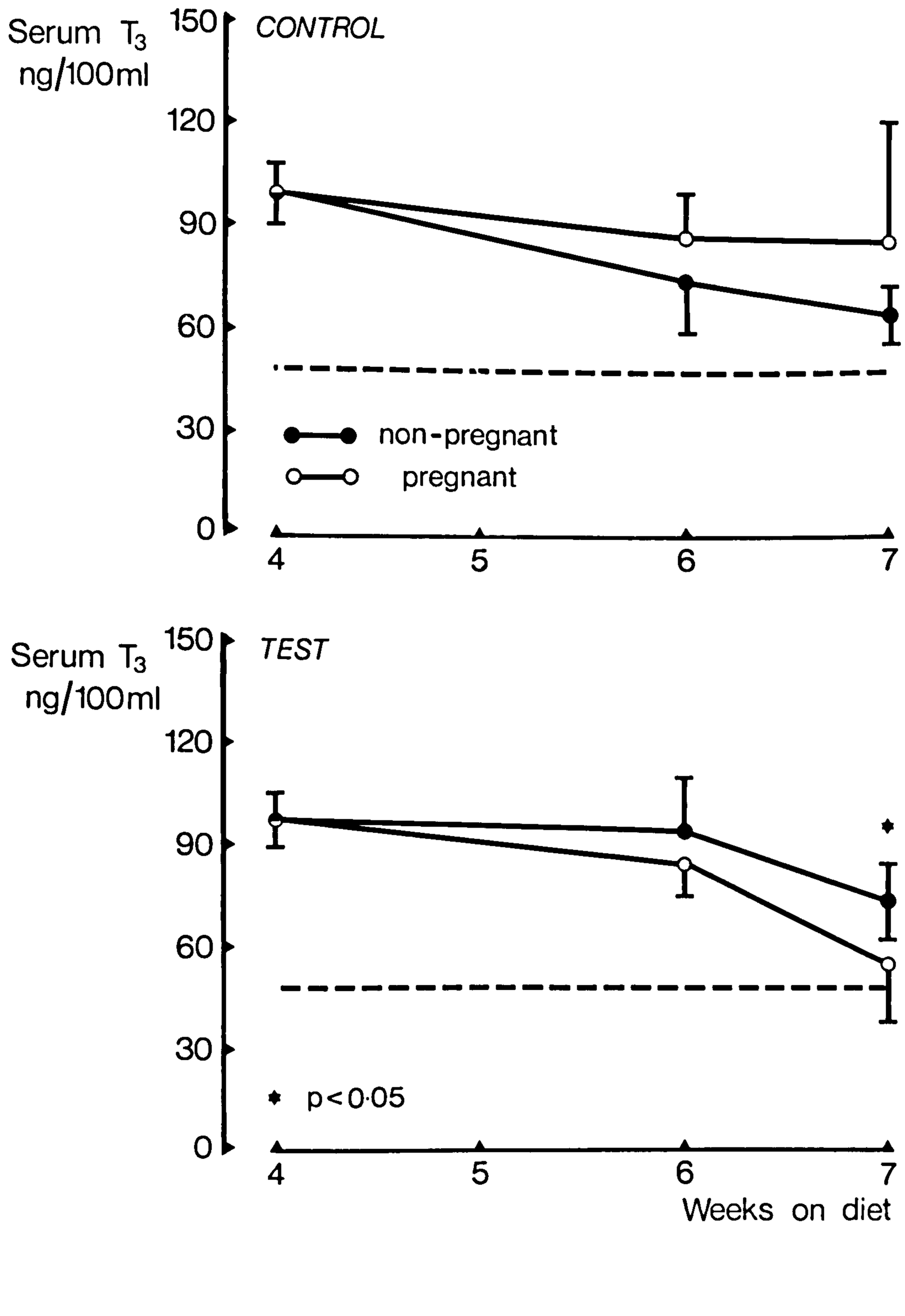
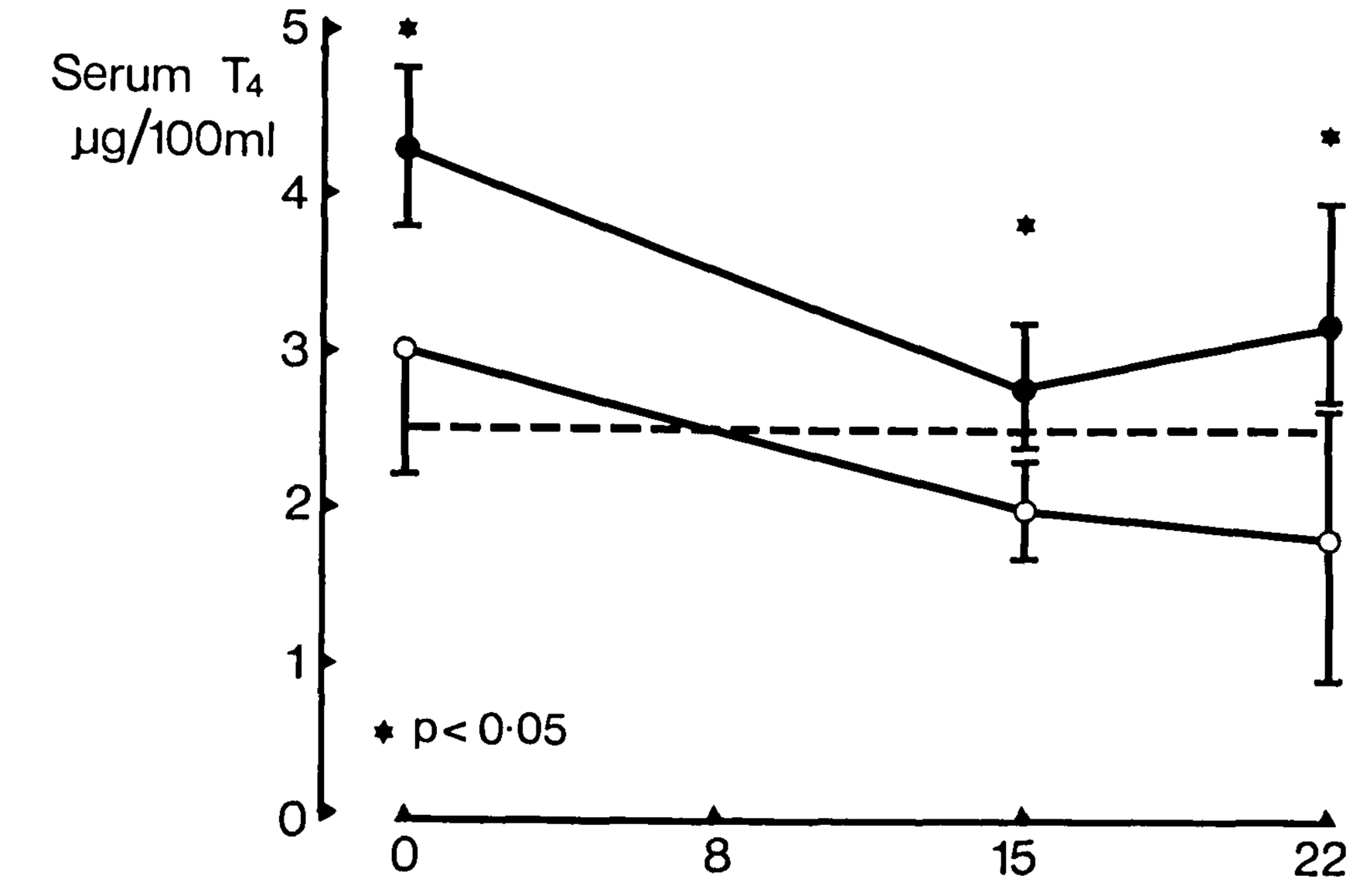

Serum T_3 ng/100ml
150
120
90
60
30
0
CONTROL
non-pregnant
pregnant
4
5
6
7
Serum T_3 ng/100ml
150
120
90
60
30
0
TEST
* p<0·05
4
5
6
7
Weeks on diet

Fig. 33

Fig. 34 Effect of mild iodine deficiency on serum T_4 and T_3 changes during pregnancy.


Serum T_4
µg/100ml
5
4
3
2
1
0
* p< 0·05
0
8
15
22

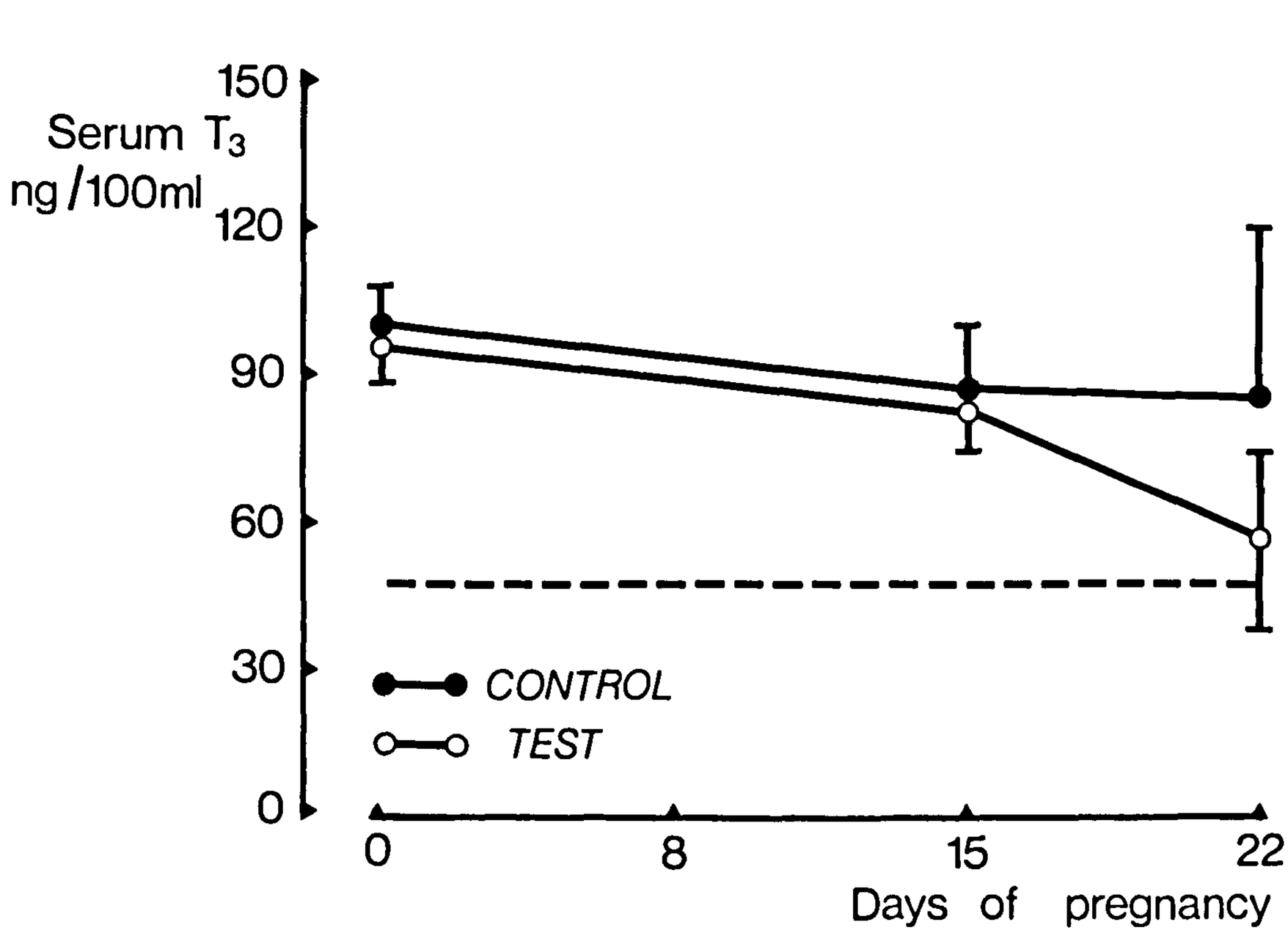

Serum T_3
ng/100ml
150
120
90
60
30
0
CONTROL
TEST
0
8
15
22
Days of pregnancy

Fig. 34

Fig. 35 Changes in serum T_4 and T_3 concentrations in mildly iodine deficient mothers during the last seven days of gestation.

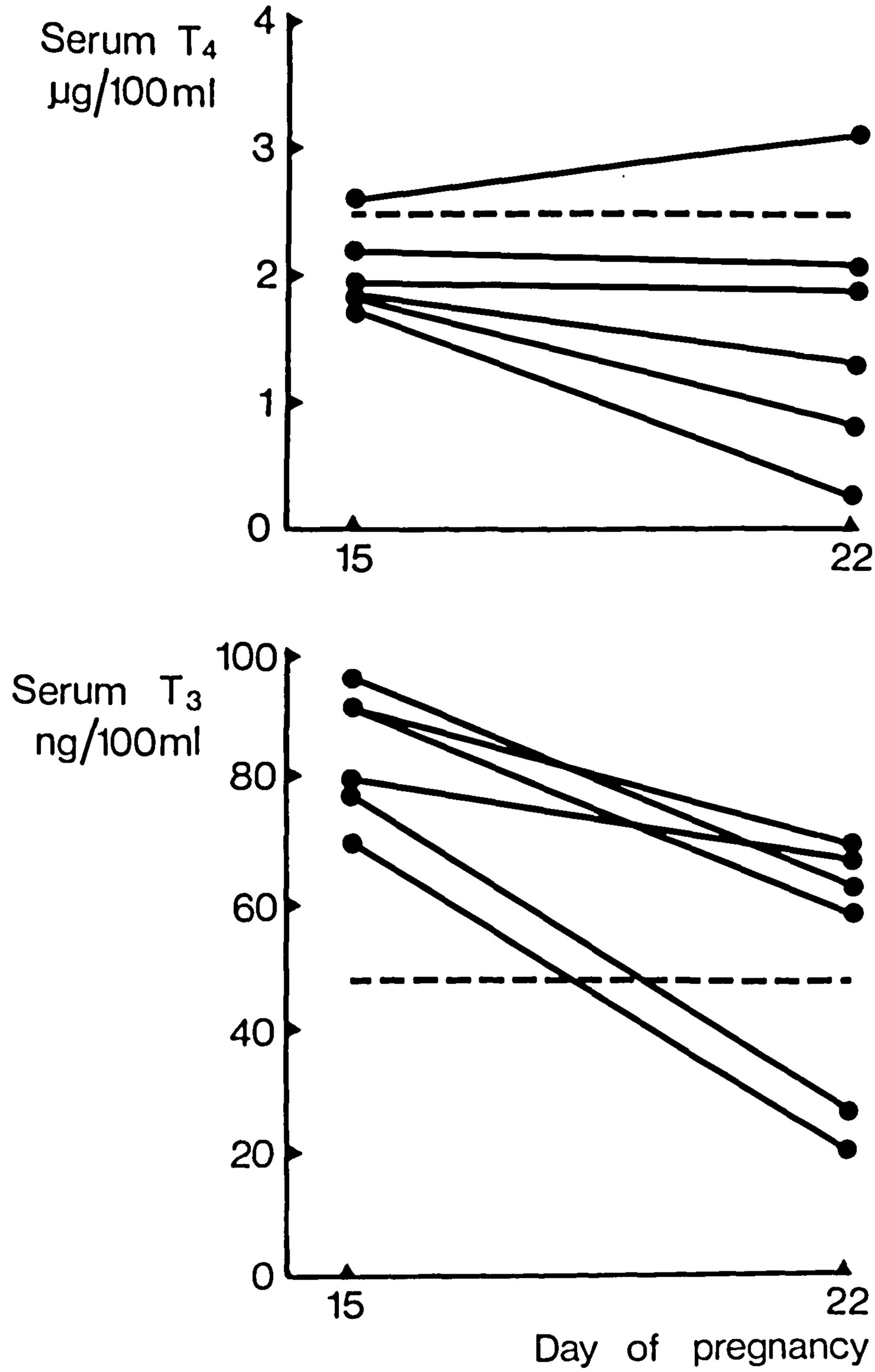

Serum T_4
µg/100ml
4
3
2
1
0
15
22
Serum T_3
ng/100ml
100
80
60
40
20
0
15
22
Day of pregnancy

Fig. 35

Fig. 36 Changes in serum T_4 concentration during pregnancy and lactation in Experiment 7.

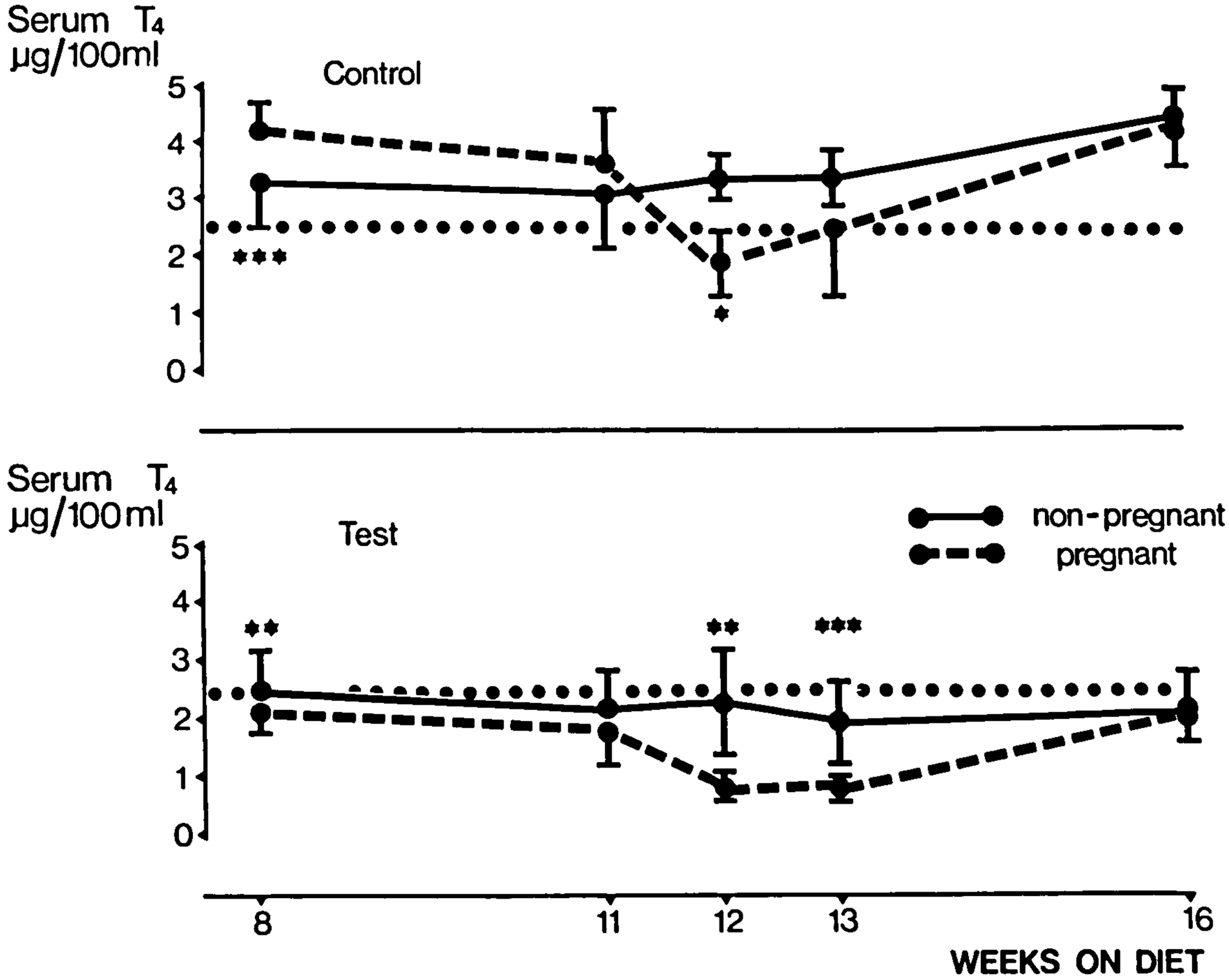

Serum T4
µg/100ml
Control
5
4
3
2
1
0
***
*
Serum T4
µg/100ml
Test
non-pregnant
pregnant
**
**
***
8
11
12
13
16
WEEKS ON DIET

Fig. 36

Fig. 37 Changes in serum T_3 concentration during pregnancy and lactation in Experiment 7.

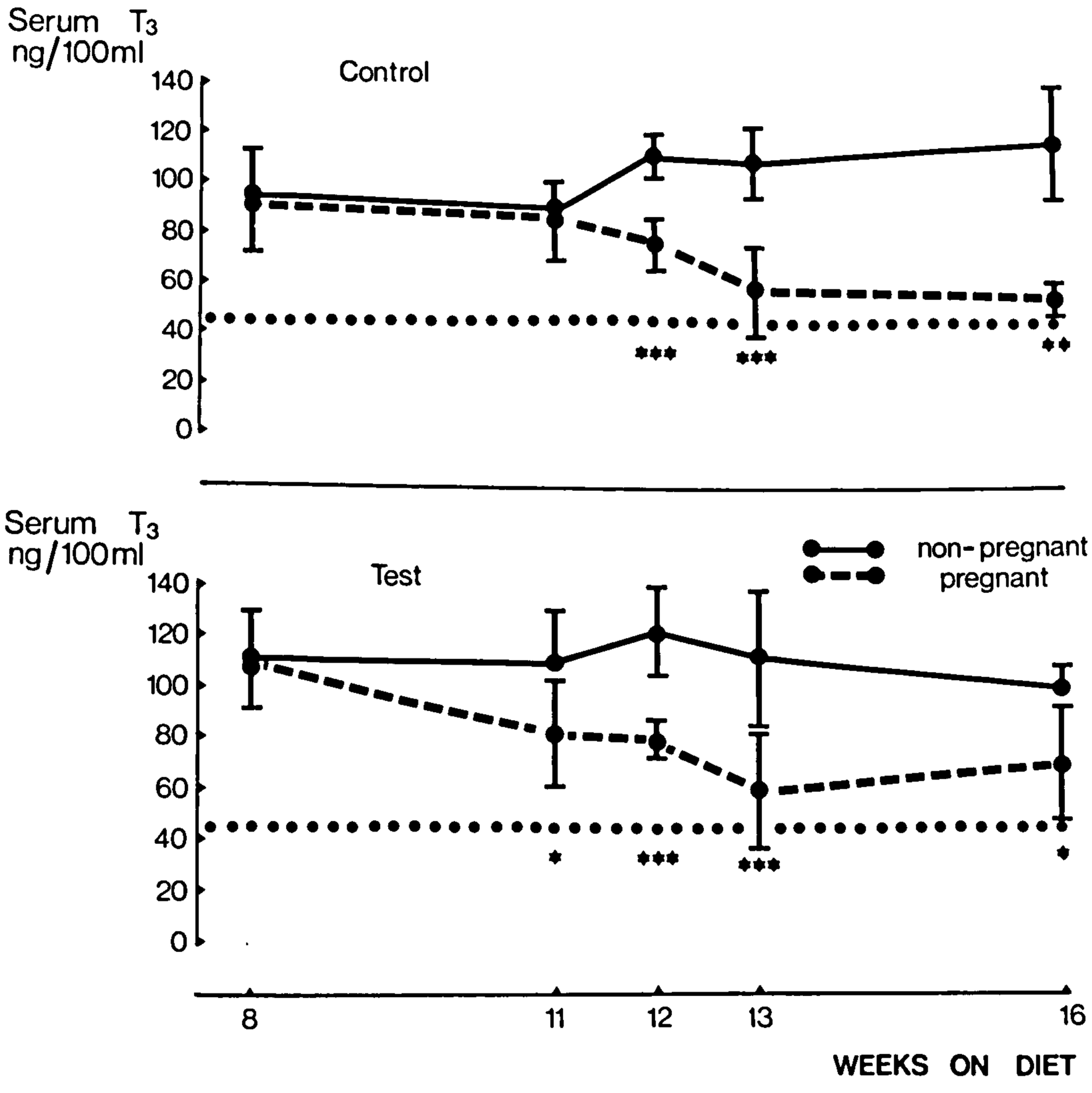

Serum T_3
ng/100ml
Control
140
120
100
80
60
40
20
0
***
***
**
Serum T_3
ng/100ml
Test
non-pregnant
pregnant
140
120
100
80
60
40
20
0
*
***
***
8
11
12
13
16
WEEKS ON DIET

Fig. 37

Fig. 38 Effect of moderate iodine deficiency on serum T_4, T_3 and T_3/T_4 ratio during pregnancy and lactation.

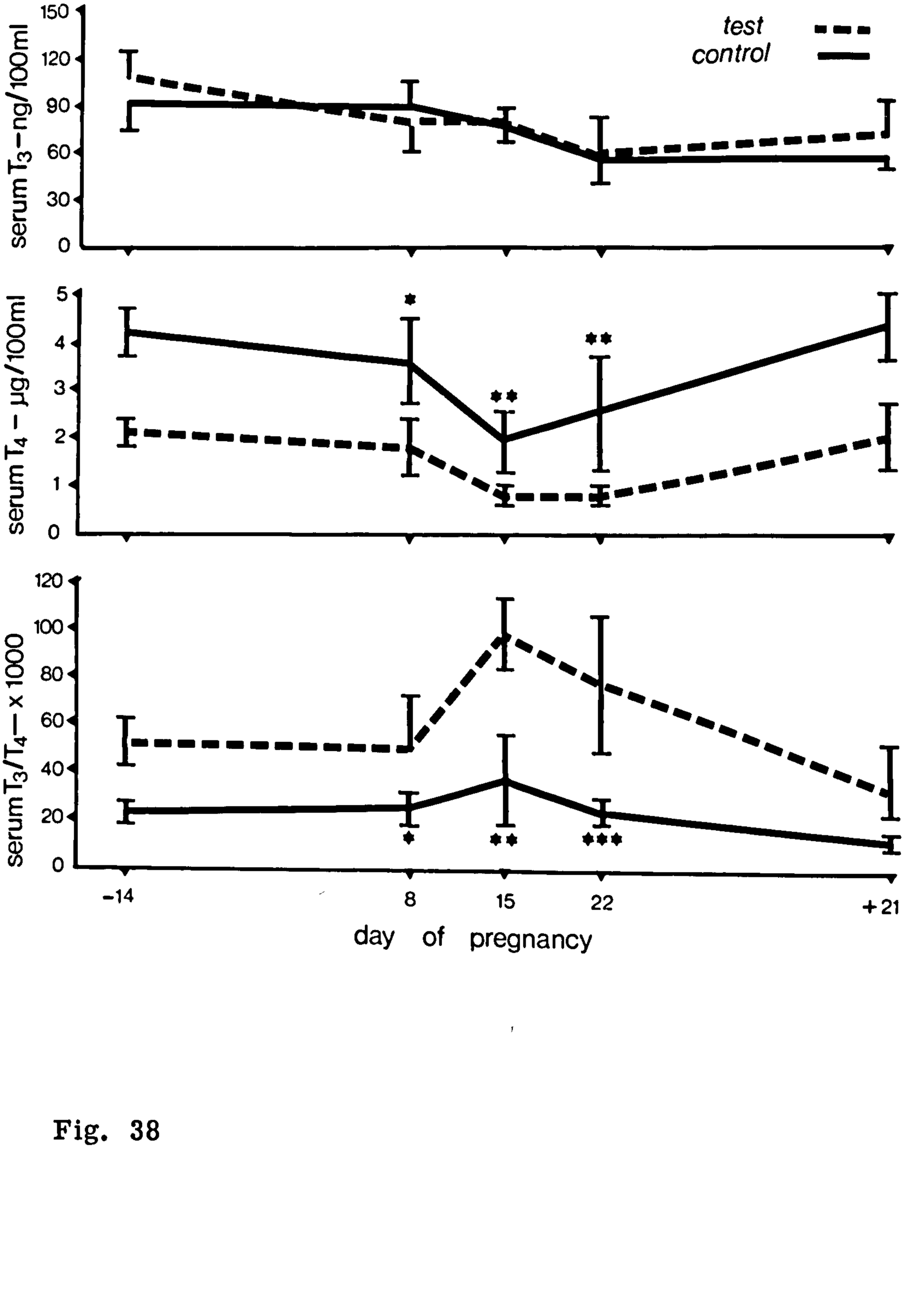

test
control
serum T_3 – ng/100ml
150
120
90
60
30
0
serum T_4 – µg/100ml
5
4
3
2
1
0
*
**
**
serum T_3/T_4 – x 1000
120
100
80
60
40
20
0
*
**
***
-14
8
15
22
+21
day of pregnancy

Fig. 38

Table 1

Effect of diet I for periods up to 4 weeks on relative thyroid weight and radioactive iodine uptake

Weeks on diet	Number of animals	RTW (mg/100 gbw)	RAIU (% injected dose)
Baseline	24	6.80 ± 1.11	4.88 ± 2.16
1	20	8.46 ± 1.59***	23.39 ± 10.21***
2	15	7.78 ± 1.61	27.72 ± 13.99***
3	15	8.78 ± 2.08**	42.58 ± 13.51***
4	18	8.55 ± 1.20***	37.98 ± 14.44***

Statistical comparison with baseline

p value: * ⟨0.05; ** ⟨0.01; *** ⟨0.001

Table 2

Effect of diet I for periods up to 4 weeks on serum thyroxine and triiodothyronine levels

Weeks on diet	Number of animals	Serum T_4 (μg/100 ml)	Serum T_3 (ng/100 ml)
Baseline	18	4.3 ± 1.3	105 ± 39
1	6	3.9 ± 0.4	99 ± 19
2	6	3.5 ± 0.6	100 ± 9
3	6	4.4 ± 1.5	103 ± 20
4	15	3.1 ± 1.0**	99 ± 16

p value: * $\langle 0.05$; ** $\langle 0.01$; *** $\langle 0.001$

Otherwise non-significant

Table 3

Effect of diet I for periods up to 24 weeks on relative thyroid weight and radioactive iodine uptake

Weeks on diet	Number of animals	RTW (mg/100 gbw)	RAIU (% injected dose)
Baseline	20	6.58 ± 0.86	1.19 ± 0.79
4	10	9.36 ± 1.18***	36.32 ± 21.97***
8	6	9.61 ± 1.31***	52.96 ± 12.38***
12	12	9.08 ± 1.53***	42.63 ± 9.33***
16	6	8.57 ± 1.00***	64.81 ± 8.24***
20	6	11.18 ± 2.80***	68.01 ± 10.20***
24	6	10.02 ± 1.05***	66.11 ± 6.50***

Table 4

Effect of diet I for periods up to 24 weeks on serum thyroxine and triiodothyronine levels

Weeks on diet	Number of animals	Serum T_4 (μg/100 ml)	Serum T_3 (ng/100 ml)
Baseline	44	4.6 ± 0.7	99 ± 19
4	12	3.8 ± 0.8**	95 ± 26
8	7	3.5 ± 0.7***	98 ± 28
12	6	2.8 ± 0.4***	92 ± 11
16	6	2.8 ± 0.4***	109 ± 21
20	6	1.9 ± 0.8***	113 ± 22
24	6	2.0 ± 0.3***	94 ± 18

Table 5

Effect of diet II for periods up to 4 weeks on relative thyroid weight and radioactive iodine uptake

Weeks on diet	Number of animals	RTW (mg/100 gbw)	RAIU (% injected dose)
Baseline	20	6.80 ± 1.11	4.88 ± 2.16
1	12	7.61 ± 1.29	24.77 ± 5.22***
2	12	7.64 ± 0.98*	34.81 ± 10.26***
3	12	10.30 ± 1.44***	43.50 ± 8.77***
4	17	8.78 ± 1.08***	43.72 ± 13.61***

Table 6

Effect of diet II for periods up to 4 weeks on serum thyroxine and triiodothyronine levels

Weeks on diet	Number of animals	Serum T_4 (μg/100 ml)	Serum T_3 (ng/100 ml)
Baseline	18	4.3 ± 1.3	105 ± 39
1	12	3.5 ± 0.6*	116 ± 39
2	12	3.9 ± 1.0	105 ± 48
3	12	3.9 ± 1.4	110 ± 24
4	12	3.2 ± 0.7**	104 ± 17

Table 7

Effect of diet II for periods up to 12 weeks on relative thyroid weight and radioactive iodine uptake

Weeks on diet	Number of animals	RTW (mg/100 gbw)	RAIU (% injected dose)
Baseline	24	6.80 ± 1.11	4.88 ± 2.16
2	12	7.64 ± 0.98*	34.81 ± 10.26***
4	17	8.78 ± 1.08***	43.72 ± 13.61***
6	6	7.64 ± 0.99	42.96 ± 12.69***
8	6	8.27 ± 1.55*	52.07 ± 20.94***
12	6	8.80 ± 1.28**	50.15 ± 13.60***

Table 8

Effect of diet II for periods up to 24 weeks on serum thyroxine and triiodothyronine levels

Weeks on diet	Number of animals	Serum T_4 (μg/100 ml)	Serum T_3 (ng/100 ml)
Baseline	18	4.3 ± 1.3	105 ± 39
2	12	3.9 ± 1.0	105 ± 48
4	12	3.2 ± 0.7*	104 ± 17
6	6	2.7 ± 0.3***	105 ± 14
8	6	2.5 ± 0.7***	101 ± 15
10	6	2.1 ± 0.1***	101 ± 24
12	8	2.2 ± 0.6***	123 ± 18
16	9	2.1 ± 0.7***	100 ± 9
20	9	2.2 ± 0.3***	123 ± 20
24	32	2.1 ± 0.6***	117 ± 23

Table 9

Comparison of effects of diets I and II on serum thyroxine levels

Weeks on diet	Serum thyroxine*		Significance of difference
	Diet I	Diet II	
4	3.8 ± 0.8	3.2 ± 0.7	n. s.
8	3.5 ± 0.7	2.5 ± 0.7	$p<0.005$
12	2.8 ± 0.4	2.2 ± 0.6	$p<0.05$
16	2.8 ± 0.4	2.1 ± 0.7	$p<0.05$
20	1.9 ± 0.8	2.2 ± 0.3	n. s.
24	2.0 ± 0.3	2.1 ± 0.6	n. s.

* expressed as μg/100 ml

n. s. = non-significant ($p>0.05$)

Table 10

Comparison of wet weight and chemical composition of forebrain in neonates and 22 day old weanling pups

	Neonate	day 22 pup	Increment
Forebrain weight (mg)	151	1035	+ 585%
Total DNA (μg)	620	844	+ 36%
Total protein (mg)	7.0	47.4	+ 577%
Protein:DNA ratio	11.4	56.3	+ 279%

Values given are means of at least 12 control animals

Table 11

Effect of LID introduced at mating on neonatal body and forebrain weights (all pups)

Male and female pups (n = 33)	Control (19)	Test (14)	Significance of difference
Body weight (g)	5.61 ± 0.69	5.91 ± 0.54	n.s.
Forebrain weight (mg)	150.79 ± 11.18	162.18 ± 6.76	$p < 0.005$
Relative forebrain weight (mg/g)	27.07 ± 1.95	27.58 ± 1.82	n.s.

Values given are means ± one S.D.

Statistical comparison using Student's t test

Table 12

Effect of LID introduced at mating on neonatal body and forebrain weights (male pups)

Male pups (n = 20)	Control (13)	Test (7)	Significance of difference
Body weight (g)	5.58 ± 0.60	6.28 ± 0.43	$p < 0.01$
Forebrain weight (mg)	149.33 ± 7.19	166.56 ± 4.73	$p < 0.001$
Relative forebrain weight (mg/g)	26.96 ± 2.04	26.59 ± 1.30	n.s.

Table 13

Effect of LID introduced at mating on DNA and protein content of neonatal forebrain

Male and female pups (n = 33)	Control (19)	Test (14)	Significance of difference
Total DNA (μg)	622 ± 71	626 ± 50	n. s.
Total protein (mg)	7.27 ± 1.39	7.22 ± 1.49	n. s.
Protein:DNA ratio	12.01 ± 3.12	11.74 ± 3.10	n. s.

Table 14

Effect of LID introduced at mating on thyroid parameters of mothers in immediate post-partum period

	Control (2)	Test (2)	% difference
Thyroid weight (mg)	11.42	20.96	+ 83
RTW (mg/100g)	4.34	8.05	+ 85
RAIU (% dose)	4.56	33.31	+ 630

Table 15

Effect of LID introduced at mating on serum thyroid hormone levels of post-partum mothers

	Control (2)	Test (2)	% difference
Serum T_4 (μg/100 ml)	3.2	2.9	-10
Serum T_3 (ng/100 ml)	120	121	0

Table 16

Effect of LID fed to mothers from 4 weeks before mating and throughout gestation on litter size and perinatal mortality

	Control	Test
Number of litters[+]	4 (5)	5 (6)
Number of pups	35 (39)	57 (58)
Average litter size	9 (8)	11 (10)
Liveborn	30	57
Stillborn	5	0
Postmature	4	1

[+]Numbers in parentheses include litters delivered by uterotomy

Table 17

Effect of LID fed to mothers from 4 weeks before mating and throughout gestation on body and forebrain weights of newborn

	Control (4)	Test (5)	% change	Significance of difference
Body weight (g)	5.76 ± 0.39	5.43 ± 0.26	-6	n.s.
Forebrain weight (mg)	156.86 ± 9.32	151.11 ± 8.11	-4	n.s.
Relative forebrain weight (mg/g)	27.40 ± 1.53	27.92 ± 1.85	+1	n.s.

Numbers in parentheses represent numbers of litters rather than pups.

Statistical comparison using unpaired Student's t test.

Table 18

Effect of LID fed to mothers from 4 weeks before mating and throughout gestation on neonatal forebrain chemical composition

	Control (4, 30)	Test (5, 57)	% change	Significance of difference
Total DNA (μg)	627 $\pm$ 21	610 $\pm$ 21	-3	n. s.
Total Protein (mg)	7.1 $\pm$ 0.6	7.0 $\pm$ 0.4	-2	n. s.
Protein:DNA (mg/mg)	11.30 $\pm$ 0.59	11.45 $\pm$ 0.61	+1	n. s.

First number in parentheses: number of litters

Second number: number of brains analysed

Table 19

Effect of iodine supplementation on thyroid function of animals fed LID for 4 weeks prior to mating

	Control (5)	Test (5)	% change*	Significance of difference
Relative thyroid weight (mg/100 g)	6.23 ± 0.79	8.87 ± 0.73	+42	$p<0.001$
4 hr RAIU (% dose)	16.58 ± 4.86	38.91 ± 15.66	+135	$p<0.02$
Serum T_4 (μg/100 ml)	4.3 ± 0.5	3.1 ± 0.9	-23	$p<0.05$
Serum T_3 (ng/100 ml)	99 ± 9	97 ± 8	-2	n.s.

*% change refers to alterations in test animals

Numbers in parentheses refer to individual animals

Table 20

Results of thyroid function tests performed after 7 weeks of LID on animals mated after 4 weeks

	Control (10)	Test (10)	% change	Significance of difference
Relative thyroid weight (mg/100 g)	5.88 ± 1.47	8.83 ± 1.61	+50	$p<0.01$
4 hr RAIU (% dose)	13.23 ± 4.67	43.41 ± 14.02	+221	$p<0.001$
Serum T_4 (μg/100 ml)	3.5 ± 0.7	2.5 ± 1.1	-29	$p<0.05$
Serum T_3 (ng/100 ml)	76 ± 28	64 ± 15	-16	n.s.

Table 21

Effect of LID on body weights of animals fed ad libitum for 10 weeks prior to mating

Weeks	0	5	10
Control (40)	168 ± 17*	240 ± 25	257 ± 18
Test (40)	159 ± 21	238 ± 21	255 ± 19
Difference	n. s.	n. s.	n. s.

*Body weights are expressed in grams

Statistical comparison by unpaired t test

n. s. = non-significant (p)0. 05)

Table 22

Effect of iodine deprivation for 8 weeks on total serum T_3 and T_4 levels and T_3/T_4 ratio

	Serum T_3 (ng/100 ml)	Serum T_4 (μg/100 ml)	$\frac{\text{Serum } T_3}{\text{Serum } T_4} \times 1000$
Control (40)	95 ± 18	3.3 ± 0.8	29.53 ± 7.61
Test (40)	111 ± 19	2.5 ± 0.7	47.99 ± 18.41
p value	<0.001	<0.001	<0.001

Numbers in parentheses refer to sera analysed

Value of p calculated by standard t test

Table 23

Comparison of total serum T_3 and T_4 levels and T_3/T_4 ratio in control and test animals selected for mating

	Serum T_3 (ng/100 ml)	Serum T_4 (μg/100 ml)	$\frac{\text{Serum } T_3}{\text{Serum } T_4} \times 1000$
Control (20)	99 ± 18	3.8 ± 0.7	27.01 ± 6.72
Test (20)	115 ± 19	2.0 ± 0.4	58.89 ± 20.29
p value	<0.001	<0.001	<0.001

Table 24

Effect of iodine supplementation on thyroid function of infertile animals fed LID for 13 weeks

	Control (5)	Test (5)	% change	Significance of difference
Relative thyroid weight (mg/100 g)	7.26 ± 2.17	10.39 ± 1.85	+43	$p<0.02$
4 hr RAIU (% dose)	3.13 ± 1.54	57.59 ± 22.76	+1740	$p<0.001$
Serum T_4 (μg/100 ml)	3.4 ± 0.5	1.9 ± 0.7	-44	$p<0.01$
Serum T_3 (ng/100 ml)	108 ± 14	112 ± 27	+4	n. s.

Table 25

Effect of LID fed to mothers from 10 weeks before mating and throughout gestation on litter size and incidence of postmaturity

	Control	Test
Number of litters	4 (5)	4 (5)
Number of pups	19 (23)	23 (30)
Average litter size	5 (5)	6 (6)
Pups born postmature	4 (17%)*	7 (23%)

*expressed as percentage of total number of pups

Table 26

Effect of LID fed to mothers from 10 weeks before mating and throughout gestation on body and forebrain weights of newborns

	Control (4, 19)	Test (4, 23)	% change	Significance of difference
Body weight (g)	6.60 ± 0.60	5.14 ± 1.31	-22	n.s.
Forebrain weight (mg)	176.57 ± 6.17	147.73 ± 23.88	-16	$p<0.05$
Relative forebrain weight (mg/g)	26.90 ± 2.25	29.53 ± 4.93	+10	n.s.

Table 27

Effect of moderate iodine deficiency on body and forebrain weights of pups born on day 22 of gestation

	Control (3, 14)	Test (3, 15)	% change	Significance of difference
Body weight (g)	6.85 ± 0.40	5.73 ± 0.70	-17	n. s.
Forebrain weight (mg)	176.87 ± 7.52	155.38 ± 22.44	-13	n. s.
Relative forebrain weight (mg/g)	25.83 ± 0.84	27.68 ± 0.76	+4	n. s.

Table 28

Effect of LID fed to mothers from 10 weeks before mating and throughout gestation on neonatal forebrain chemical composition

	Control (4, 19)	Test (4, 23)	% change	Significance of difference
Total DNA (μg)	581 $\pm$ 11	562 $\pm$ 20	-3	n. s.
Total protein (mg)	7.5 $\pm$ 0.4	6.0 $\pm$ 1.1	-20	$p<0.05$
Protein:DNA (mg/mg)	12.87 $\pm$ 0.61	10.61 $\pm$ 1.91	-18	n. s.

Table 29

Effect of moderate iodine deficiency on forebrain content of DNA and protein in pups born on day 22 of gestation

	Control (3, 14)	Test (3, 15)	% change	Significance of difference
Total DNA (μg)	582 $\pm$ 14	569 $\pm$ 18	-3	n. s.
Total protein (mg)	7.5 $\pm$ 0.5	6.5 $\pm$ 0.3	-14	$p < 0.05$

Table 30

Effect of moderate iodine deficiency on cell size in forebrains of pups born on day 22 of gestation

	Control (3, 14)	Test (3, 15)	% change	Significance of difference
Protein:DNA (mg/mg)	12.87 ± 0.75	11.49 ± 0.90	-11	n.s.
Weight:DNA (mg/μg)	304 ± 6	274 ± 38	-10	$p < 0.001$

Table 31

Effect of LID fed for 24 weeks on serum T_4 and T_3 concentrations

	Control (30)	Test (30)	Significance of difference
Serum T_4 (μg/100 ml)	4.1 ± 1.0	2.1 ± 0.6	$p<0.001$
Serum T_3 (ng/100 ml)	114 ± 21	117 ± 23	n. s.

Table 32

Comparison of levels of serum T_4 and T_3 in animals selected for mating

	Control (10)	Test (10)	Significance of difference
Serum T_4 ($\mu g/100$ ml)	4.7 ± 0.4	1.5 ± 0.1	$p < 0.001$
Serum T_3 (ng/100 ml)	119 ± 32	109 ± 16	n. s.

Table 33

Effect of LID fed for six months prior to mating and throughout gestation on litter size and perinatal mortality

	Control	Test
No. of litters	3	3
Average litter size	7	10
No. of pups	21	29
Born alive	9 (63%)	0 (0%)
Stillborn	11 (52%)	10 (35%)
Died in utero	1 (5%)	19 (65%)

Table 34

Effect of severe prenatal iodine deficiency on body and forebrain growth and development

	Control (3, 12)	Test (3, 29)	% change	Significance of difference
Body weight (g)	6.55 ± 0.46	6.30 ± 0.90	-4	n.s.
Forebrain weight (mg)	176.68 ± 8.23	196.60 ± 12.81	+11	n.s.
Relative forebrain weight (mg/g)	26.94 ± 0.97	31.40 ± 3.23	+16	n.s.

Table 35

Effect of severe prenatal iodine deficiency on neonatal forebrain chemical composition

	Control (3, 21)	Test (3, 29)	% change	Significance of difference
Total DNA (μg)	548 $\pm$ 29	580 $\pm$ 85	+6	n. s.
Total Protein (mg)	9.21 $\pm$ 0.23	8.02 $\pm$ 0.39	-13	$p<0.01$
Protein:DNA (mg/mg)	16.85 $\pm$ 1.25	13.83 $\pm$ 2.19	-18	n. s.

Table 36

Effect of severe iodine deficiency on thyroid function of animals sacrificed in the immediate post-partum period

	Control (3)	Test (3)	Significance of difference
Serum T_4 (μg/100 ml)	2.7 ± 0.8	1.00 ± 0.02	$p < 0.025$
Serum T_3 (ng/100 ml)	57 ± 18	33 ± 13	n. s.
RTW (mg/100 g)	6.69 ± 0.44	11.41 ± 1.26	$p < 0.005$
RAIU (% dose)	2.70 ± 0.60	41.63 ± 17.14	$p < 0.02$

Table 37

Effect of combined prenatal and postnatal iodine deficiency on body weights of pups during the period of maternal lactation

	Control	Test	% change	Significance of difference
Day 0	6.12 ± 0.44 (24)	5.73 ± 0.38 (24)	-6	$p < 0.001$
Day 7	11.18 ± 1.66 (19)	12.54 ± 1.04 (20)	+12	$p < 0.005$
Day 14	20.37 ± 3.93 (19)	22.32 ± 0.99 (20)	+9	$p < 0.05$
Day 21	28.34 ± 5.91 (19)	31.09 ± 2.91 (20)	+9	n. s.

The numbers of pups are in parentheses

Values given are means ± 1 S.D.

Table 38

Effect of combined prenatal and postnatal iodine deficiency on body and forebrain weights of 22 day old weanlings

Male and female pups (n = 39)	Control (19)	Test (20)	% change	Significance of difference
Body weight (g)	30.63 ± 6.87	32.46 ± 2.94	+5	n. s.
Forebrain weight (mg)	1031.64 ± 67.45	1009.84 ± 47.94	-3	n. s.
Relative forebrain weight (mg/g)	35.02 ± 6.44	31.27 ± 2.09	-11	$p < 0.025$

Table 39

Effect of male or female sex on body and forebrain weights of test pups at 22 postnatal days

	Male (14)	Female (6)	% change	Significance of difference
Body weight (g)	33.36 ± 2.76	30.36 ± 2.35	-9	$p < 0.025$
Forebrain weight (mg)	1030.20 ± 38.87	963.34 ± 30.61	-7	$p < 0.001$
Relative forebrain weight (mg/g)	31.04 ± 2.28	31.79 ± 15.76	+2	n.s.

Table 40

Effect of combined prenatal and postnatal iodine deficiency on body and forebrain weights of 22 day old male weanlings

	Control (12)	Test (14)	% change	Significance of difference
Body weight (g)	30.34 ± 6.81	33.36 ± 2.76	+9	n. s.
Forebrain weight (mg)	1043.63 ± 56.32	1030.20 ± 38.87	-2	n. s.
Relative forebrain weight (mg/g)	35.76 ± 6.73	31.04 ± 2.28	-14	$p < 0.05$

Table 41

Effect of combined prenatal and postnatal iodine deficiency on forebrain content of DNA and protein in 22 day old weanlings

	Control (19)	Test (20)	% change	Significance of difference
Total DNA (μg)	853 ± 91	836 ± 76	-2	n. s.
Total Protein (mg)	47.01 ± 10.01	47.36 ± 10.62	+1	n. s.
Protein:DNA (mg/mg)	55.27 ± 10.06	57.32 ± 15.08	+4	n. s.

Table 42

Effect of combined prenatal and postnatal iodine deficiency on forebrain content of DNA and protein in 22 day old male weanlings

	Control (12)	Test (14)	% change	Significance of difference
Total DNA (μg)	866 $\pm$ 78	822 $\pm$ 63	-5	n. s.
Total Protein (mg)	48.24 $\pm$ 11.29	47.43 $\pm$ 11.82	-2	n. s.
Protein:DNA (mg/mg)	55.68 $\pm$ 11.38	58.21 $\pm$ 16.37	+2	n. s.

Table 43

Effect of male or female sex on forebrain content of DNA and protein in test pups at 22 postnatal days

	Male (14)	Female (6)	% change	Significance of difference
Total DNA (μg)	822 $\pm$ 63	867 $\pm$ 99	+5	n. s.
Total Protein (mg)	47.43 $\pm$ 11.82	47.19 $\pm$ 8.11	-1	n. s.
Protein:DNA (mg/mg)	58.21 $\pm$ 16.37	55.25 $\pm$ 12.68	-6	n. s.

Table 44

Effect of LID for 16 weeks and lactation during 3 weeks on in vitro thyroid function at 21 postnatal days

	Control (3)	Test (3)	Significance of difference
Thyroid weight (mg)	14.81 ± 1.91	22.53 ± 7.78	$p < 0.005$
Relative thyroid weight (mg/100 g)	5.18 ± 0.28	7.72 ± 1.85	n.s.
RAIU (% dose)	1.57 ± 1.03	24.86 ± 9.35	$p < 0.001$

Table 45

Effect of moderate iodine deficiency on serum thyroxine concentration before and after successful pregnancy

	Control (3)	Test (3)	% change	Significance of difference
Before mating	4.2 ± 1.0*	2.3 ± 0.05	-45	$p<0.025$
Post-partum	1.9 ± 0.6	0.8 ± 0.3	-57	$p<0.05$
At weaning	4.3 ± 0.7	2.0 ± 0.7	-53	$p<0.05$

*Values expressed as μg/100 ml

Table 46

Effect of moderate iodine deficiency on serum triiodothyronine concentration before and after successful pregnancy

	Control (3)	Test (3)	% change	Significance of difference
Before mating	101 ± 23*	106 ± 15	+5	n. s.
Post-partum	46 ± 13	62 ± 12	+35	n. s.
At weaning	54 ± 6	70 ± 22	+30	n. s.

*Values expressed as ng/100 ml

Table 47

Effects of pregnancy and lactation on serum thyroid hormone concentrations of control mothers

	Serum T_3 (ng/100 ml)	Serum T_4 (μg/100 ml)	$\frac{\text{Serum } T_3}{\text{Serum } T_4} \times 1000$
Non-pregnant (5)	117 ± 24	4.6 ± 0.6	25.96 ± 6.78
21 days post-partum (3)	54 ± 6	4.3 ± 0.7	12.82 ± 3.15
p value	<0.005	n. s.	<0.01

Table 48

Effects of pregnancy and lactation on serum thyroid hormone concentrations of test mothers

	Serum T_3 (ng/100 ml)	Serum T_4 (μg/100 ml)	$\frac{\text{Serum } T_3}{\text{Serum } T_4} \times 1000$
Non-pregnant (9)	100 ± 9	2.1 ± 0.7	53.44 ± 18.80
21 days post-partum (3)	70 ± 22	2.0 ± 0.7	36.74 ± 15.26
p value	<0.05	n. s.	n. s.

Table 49

Effect of combined prenatal and postnatal iodine deficiency on thyroid function in the 22 day old weanling

	Control	Test	Significance
Thyroid weight (mg)	5.49 ± 2.65 (7)	7.41 ± 1.08 (9)	n. s.
Relative thyroid weight (mg/100 g)	15.88 ± 5.94 (7)	22.12 ± 1.99 (9)	$p<0.02$
4 hr RAIU (% dose)	1.39 ± 0.78 (10)	35.79 ± 8.95 (9)	$p<0.01$
Serum T_4 (μg/100 ml)	5.0 ± 1.5 (9)	5.0 ± 0.9 (11)	n. s.
Serum T_3 (ng/100 ml)	75 ± 35 (9)	121 ± 32 (11)	$p<0.01$

Table 50

Comparison of thyroid function in control weanlings and their mothers at time of weaning

	Mothers (3)	Weanlings (9)	Significance of difference
Serum T_4 (μg/100 ml)	4.3 $\pm$ 0.7	5.0 $\pm$ 1.5	n. s.
Serum T_3 (ng/100 ml)	54 $\pm$ 6	75 $\pm$ 35	n. s.
4 hr RAIU (% dose)	1.57 $\pm$ 1.03	1.39 $\pm$ 0.78	n. s.

Table 51

Comparison of thyroid function in test weanlings and their mothers at time of weaning

	Mothers (3)	Weanlings (11)	Significance of difference
Serum T_4 (μg/100 ml)	2.0 ± 0.4	5.0 ± 0.9	$p < 0.001$
Serum T_3 (ng/100 ml)	70 ± 22	121 ± 32	$p < 0.01$
4 hr RAIU (% dose)	24.86 ± 1.85	35.79 ± 8.95	n.s.

Table 52

Effects of pregnancy and mild iodine deficiency on maternal serum T_4 concentrations ($\mu g/100$ ml)

	Control (10)	Test (10)	Significance of difference
Non-pregnant	3.9 ± 1.1 (5)	3.3 ± 0.8 (4)	n. s.
15-day pregnant	2.8 ± 0.4 (5)	2.0 ± 0.3 (6)	$p < 0.005$
p value	n. s.	< 0.02	
Non-pregnant	4.0 ± 0.7 (5)	3.4 ± 0.7 (4)	$p < 0.025$
<6 hr post-partum	3.2 ± 0.8 (5)	1.8 ± 0.9 (6)	$p < 0.025$
p value	n. s.	< 0.02	

Numbers in parentheses refer to sera analysed

Table 53

Effects of pregnancy and mild iodine deficiency on maternal serum T_3 concentrations (ng/100 ml)

	Control (10)	Test (10)	Significance of difference
Non-pregnant	75 ± 18 (5)	93 ± 16 (4)	n. s.
15-day pregnant	87 ± 13 (5)	84 ± 10 (6)	n. s.
p value	n. s.	n. s.	
Non-pregnant	65 ± 8 (5)	73 ± 11 (4)	n. s.
⟨6 hr post-partum	85 ± 36 (5)	56 ± 18 (6)	n. s.
p value	n. s.	⟨0.025	

Table 54

Effect of pregnancy on serum T_3, T_4 and T_3/T_4 ratio in control animals fed LID with iodine supplements for 10 weeks prior to mating

	Serum T_3 (ng/100 ml)	Serum T_4 (μg/100 ml)	$\frac{\text{Serum } T_3}{\text{Serum } T_4} \times 1000$
Non-pregnant	90 ± 11 (4)	3.1 ± 1.0 (4)	30.18 ± 7.92 (4)
8-day pregnant	87 ± 18 (3)	3.6 ± 0.9 (3)	25.01 ± 6.77 (3)
p value	n.s.	n.s.	n.s.
Non-pregnant	111 ± 9 (4)	3.4 ± 0.4 (4)	34.78 ± 6.36 (9)
15-day pregnant	76 ± 10 (5)	1.9 ± 0.6 (5)	37.20 ± 18.79 (5)
p value	$<$0.001	$<$0.005	n.s.
Non-pregnant	108 ± 14 (5)	3.4 ± 0.5 (5)	31.27 ± 3.97 (5)
$<$6 hr post-partum	57 ± 18 (8)	2.5 ± 1.2 (8)	24.17 ± 4.76 (8)
p value	$<$0.001	n.s.	$<$0.02

Table 55

Effect of pregnancy on serum T_3, T_4 and T_3/T_4 ratio in test animals fed LID for 10 weeks prior to mating

	Serum T_3 (ng/100 ml)	Serum T_4 (μg/100 ml)	$\frac{\text{Serum } T_3}{\text{Serum } T_4} \times 1000$
Non-pregnant	110 ± 21 (5)	2.2 ± 0.6 (5)	42.66 ± 12.74 (5)
8-day pregnant	81 ± 21 (6)	1.8 ± 0.6 (6)	49.64 ± 22.30 (6)
p value	⟨0.05	n.s.	n.s.
Non-pregnant	123 ± 18 (8)	2.3 ± 0.9 (8)	43.54 ± 15.19 (8)
15-day pregnant	79 ± 8 (3)	0.8 ± 0.2 (3)	97.92 ± 14.89 (3)
p value	⟨0.001	⟨0.005	⟨0.001
Non-pregnant	112 ± 27 (8)	1.9 ± 0.7 (8)	48.75 ± 16.83 (8)
⟨6 hr post-partum	59 ± 23 (9)	0.8 ± 0.2 (9)	77.31 ± 28.18 (9)
p value	⟨0.001	⟨0.001	⟨0.025

Table 56

Effect of moderately severe iodine deficiency on changes in maternal serum T_4 during pregnancy and lactation

	Control	Test	Significance
Before mating	4.2 ± 0.5 (8)	2.1 ± 0.3 (9)	$p<0.001$
day 8	3.6 ± 0.9 (3)	1.8 ± 0.6 (6)	$p<0.02$
day 15	1.9 ± 0.6 (5)	0.8 ± 0.2 (3)	$p<0.01$
day 22	2.5 ± 1.2 (8)	0.8 ± 0.2 (9)	$p<0.005$
At weaning	4.3 ± 0.7 (3)	2.0 ± 0.7 (3)	$p<0.02$

Sera analysed represented by numbers in parentheses

Statistical comparison by unpaired t test

Table 57

Effect of moderately severe iodine deficiency on changes in maternal serum T_3 during pregnancy and lactation

	Control	Test	Significance
Before mating	92 ± 20 (8)	110 ± 18 (9)	n. s.
day 8	87 ± 18 (3)	81 ± 21 (6)	n. s.
day 15	76 ± 10 (5)	79 ± 8 (3)	n. s.
day 22	57 ± 18 (8)	59 ± 23 (9)	n. s.
At weaning	54 ± 6 (3)	70 ± 22 (3)	n. s.

Table 58

Effect of moderately severe iodine deficiency on changes in maternal serum T_3/T_4 ratio (x 1000) during pregnancy and lactation

	Control	Test	Significance
Before mating	22.07 ± 4.60 (8)	52.43 ± 10.26 (9)	$p<0.001$
day 8	25.01 ± 6.77 (3)	44.64 ± 22.30 (6)	$p<0.05$
day 15	37.20 ± 18.79 (6)	97.92 ± 14.89 (3)	$p<0.005$
day 22	24.17 ± 4.76 (8)	77.31 ± 28.18 (9)	$p<0.001$
At weaning	12.82 ± 3.15 (3)	36.74 ± 11.26 (3)	$p<0.05$

Table 59

Changes in maternal serum T_4 concentrations during the second week of pregnancy

	day 8 of gestation	day 15 of gestation	% change	Significance of difference
Control	3.6 ± 0.9 (3)	1.9 ± 0.6 (5)	-47	$p<0.001$
Test	1.8 ± 0.6 (6)	0.8 ± 0.2 (3)	-56	$p<0.01$

Values expressed as $\mu g/100$ ml

Table 60

Changes in maternal serum T_3 concentrations during the last seven days of pregnancy

	day 15 of gestation	⟨6 hr post-partum	% change	Significance of difference
Control	76 ± 10 (5)	57 ± 18 (8)	-25	p⟨0.05
Test	79 ± 8 (3)	59 ± 23 (9)	-25	p⟨0.05

Values expressed as ng/100 ml

Table 61

Changes in maternal serum T_4 concentrations during the period of lactation

	day 22 of gestation	postnatal day 21	% change	Significance of difference
Control	2.5 ± 1.2 (8)	4.3 ± 0.7 (3)	+72	$p<0.02$
Test	0.8 ± 0.2 (9)	2.0 ± 0.4 (3)	+150	$p<0.001$

Values expressed as $\mu g/100$ ml